VOL. 2 FASC. 5

PSYCHOPHARMACOLOGIA

SPRINGER-VERLAG / BERLIN · GÖTTINGEN · HEIDELBERG

(Postverlagsort Berlin · 19. 10. 1961)

Die Forschung über die Wirkungen und Wirkungsweisen psychotroper Substanzen hat in den letzten Jahren einen unerhörten Aufschwung genommen. Was vordem nur ein erwünschtes Ziel war, ist zu einer neuen Wissenschaft geworden: Psychopharmakologie. Da eine fruchtbare Analyse und Synthese ihrer Probleme nur durch Zusammenarbeit aller Grundfächer (Pharmakologie, Neurochemie, Neurophysiologie, Neurologie, Psychologie und Psychiatrie) möglich wird, ist die Psychopharmakologie eine verbindende, integrierende Forschungsdisziplin. Die ständig anwachsende Literatur dieses komplexen Arbeitsgebietes ist jedoch bisher zwangsläufig über zahlreiche Zeitschriften verstreut, da es bis heute kein Spezialorgan gab, das sich ausschließlich der Psychopharmakologie widmet. Diesem dringenden Bedürfnis zu begegnen, hat sich eine Gruppe von Vertretern der verschiedenen Arbeitsrichtungen der Psychopharmakologie entschlossen, eine neue Zeitschrift „Psychopharmacologia" zu gründen. In ihr sollen die bedeutenden Fortschritte dieses Arbeitsgebietes durch Veröffentlichung experimenteller und klinischer Originalarbeiten, Übersichten der neuesten Literatur sowie kurzer Originalmitteilungen zusammengefaßt werden.

Recent years have witnessed an unprecedented advance in research on the action and effects of psychotropic drugs, and what, formerly, was just a distant goal, has now evolved into a new branch of science: psychopharmacology. As, however, any fruitful analysis and synthesis of its problems can only be attained with the aid of the complete scale of basic sciences (pharmacology, neurochemistry, neurophysiology, neurology, psychology and psychiatry), psychopharmacology constitutes an integrating discipline of research. Owing to the lack of an organ devoted especially to psychopharmacology, the constantly increasing literature pertaining to this complex field of activity has hitherto of necessity been scattered among various periodicals. In order to overcome this drawback, a group of representatives of the various psychopharmacologic sections have engaged in editing a journal, "Psychopharmacologia", in which the publication of original experimental and clinical papers, reviews of recent literature and short original notices will provide a comprehensive survey of the important progress which is being actually achieved in this field of science.

Ces dernières années ont vu un développement sans précédent dans la recherche des effets et du mode d'action des substances psychotropes sur le «Comportement» et ont fait naître une nouvelle science: la Psychopharmacologie. Comme ces problèmes ne peuvent être résolus que par la collaboration des disciplines de base telles que la pharmacologie, la neurochimie, la neurophysiologie, la psychologie et la psychiatrie, la psychopharmacologie est devenue un champ de recherche de première importance. Cependant la littérature toujours croissante en ce domaine de recherche est forcément disséminée dans de nombreux périodiques, puisqu'il n'existe pas encore de journal exclusivement consacré à la psychopharmacologie. Pour répondre à ce pressant besoin un groupe de représentants des diverses disciplines de la psychopharmacologie s'est mis en devoir de rédiger un nouveau journal dans lequel seraient rassemblés les progrès importants de ce domaine, par la publication d'ouvrages originaux expérimentaux et cliniques, ainsi que des rapports sur des questions actuelles.

Richtlinien für die Mitarbeiter siehe am Schluß des Heftes. — Directions to Authors are given at the end of this number. — Directives destinées aux auteurs, voir à la fin du fascicule.

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Psychopharmacologia

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World Federation of Neurology

Commission of Neurochemistry. Preliminary Announcement

International Symposium

on

ENZYMIC ACTIVITY IN THE CENTRAL NERVOUS SYSTEM

Göteborg, Sweden

June 18th—21st, 1962.

An International Symposium will take place in Göteborg, Sweden, from June 18th to 21st, 1962 under the auspices of the Neurochemical Commission of the World Federation of Neurology. The subject will be the enzymic activity of the central nervous system and will include the following topics:

1. Enzymic mechanisms in amino acid and protein metabolism.
2. Enzymic mechanisms in lipid metabolism.
3. Localisation and distribution of enzymic activity.
4. Enzymology of energetics and ionic movement.
5. Enzymic activity related to neural function.
6. Enzymic changes in disease.

This meeting is at present open to all interested in the field but for administrative reasons the number attending will be limited. Emphasis will be placed on short, original communications and it is hoped that there will be ample opportunity for exchange of views between workers in the clinical fields and those in the basic sciences. The registration fee will be $ 15 (£ 5 5 s. Od. sterling), payable on registration; the closing date for this is March 1st, 1962.

Further information will be made available shortly and those interested in attending and/or reading a paper should communicate with one of the secretaries. Those wishing to present a paper will have to submit an abstract in English, French, or German of not more than 300 words to one of the secretaries before December 31st, 1961. All abstracts should be accompanied by a registration form. The Organizing Committee will select the papers to be included in the programme and they reserve the right to have any paper summarised by a suitable reporter or read by title only.

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Psychopharmacologia 2, 297—303 (1961)

Original Investigations · Originalarbeiten · Travaux originaux

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Visual Illusion, Tactile Sensibility and Reaction Time under LSD-25

By
ALLAN E. EDWARDS and SIDNEY COHEN

(Received June 14, 1961)

One of the most interesting aspects of the lysergic acid diethylamide (LSD-25)[1] experience is the sensory changes that are mentioned by most subjects receiving one to two mcg/kilo. The alterations are variously described as an intensification of the viewed object, increased brilliancy and saturation of color, enhanced perception of depth and texture, and various somaesthetic sensations. Illusions, for example, the movement and change of fixed forms are often reported. The literature pertaining to LSD-25 is, in general, heavily loaded with subjective reports of these sensory alterations.

The psychophysiological mechanism of LSD-25 action has not been identified; but ABRAMSON (1955) conducted a series of descriptive experiments which seems best interpreted as a central impairment of sensory modalities. CARLSON (1958) demonstrated a rise in the brightness threshold under LSD-25, and KRILL (1960) expanded these results by using the electroretinogram (ERG) as the response measure. As a result of both the introspective reports and of the basic experimental researches cited above, it seemed worthwhile to gain additional orderly data on a broad sample of both sensory and performance tasks from a reasonable sample of subjects in order to better delineate the effects of LSD-25. The measurements taken were selected according to three criteria: 1. Classic, easily replicated. 2. Simple responses required by the Ss. 3. Diverse sampling of sensory qualities.

Subjects. Fifteen volunteer unpaid subjects, four women and eleven men from 24 to 48 years of age with an average educational level of two years of college were given 125 mcg of LSD-25 in tablet form. They were permitted to rest quietly or listen to music for three hours. During the next sixty minutes the required testing was conducted. The third and fourth hours following oral LSD-25 ingestion is considered to be the

[1] The LSD-25 used in this study was donated by Mr. HARRY ALTHOUSE, Sandoz Pharmaceuticals, San Francisco, California.

period of maximal activity. The usual precautions to safeguard the subjects were taken (COHEN, 1960). The subjects were divided into two groups, both groups were tested on two occasions at intervals of three to seven days. Group I was tested first under LSD-25, later under non-drug (control) conditions. Group II was tested under control conditions first, followed by LSD-25 testing on the second occasion. Twelve sensorimotor measurements were taken from the subjects, the order of measurements being varied randomly between subjects. For all measurements the differences between Groups I and II were examined within each by an Analysis of Variance. In all cases this order effect was found to be non-significant ($p > 0.30$).

Reaction time. The subject was seated at a table before a black panel containing one green and two red jewel lights forming an equilateral triangle with the lights three inches apart. A doorbell buzzer was behind the panel and two telegraph keys were on the table at subject's left and right. With the exception of the disjunctive test, the subject always used his preferred hand. He was instructed: "When E says 'ready', you are to depress the key(s); when the appropriate stimulus comes on, you are to release the key as quickly as possible." The time required for the subject to release the key after the appropriate stimulus was presented was measured by a standard electric timer in milliseconds (ms). The warning signal "Ready" was presented irregularly between one and five seconds before the appropriate stimulus for each trial. Four practice trials were allowed, and ten measurements were taken on each test.

Test I: Reaction time, simple, light. The green jewel light was the appropriate stimulus.

Test II: Reaction time, disjunctive, light. Subject placed his left hand on the left key and his right hand on the right key. The ipsilateral red lights were the appropriate stimuli, that is, the subject responded to the left light with his left hand and to the right light with his right hand. The left and right lights were presented five times each in a random sequence.

Test III: Reaction time, discrimination, light. The subject was instructed to respond only to the left red jewel light as the appropriate stimulus. The green light, the right red light, the buzzer, and the appropriate stimulus were presented in a random sequence.

Test IV: Reaction time, simple, buzzer. The buzzer was the appropriate stimulus.

Table 1 comprises the obtained results. Tests I, II and III were evaluated by a Lindquist (1953), Type VI Analysis of Variance Test IV by a Type I Analysis of Variance.

Table 1. *Reaction times*

Test	N	Mean time (ms)		p
		Control	LSD-25	
I Simple, Light	15	234.47	259.53	<0.01
II Disjunctive, Light	15	239.93	260.53	<0.01
III Discriminative, Light	15	240.60	264.13	<0.01
		Relative Time Units [1]		
IV Simple, Buzzer	8	44.38	54.88	<0.14

[1] Electric timer connected in series with buzzer interrupting clock time by a constant but unassessed amount.

The uniform lengthening of reaction times is different from the results reported by ABRAMSON (1955). He administered 50 and 100 mcg of LSD-25 to his subjects and did not obtain significant changes in their reaction times with a similar task. There was, however, a trend apparent in his data suggesting longer reaction times with larger amounts of LSD-25. Our data can then be considered as an extension of his work reflecting the difference in dosage.

Color detection. Subject was seated ten feet from a translucent light filter 12.5 cm. in diameter surrounded by a black panel. Behind the filter were red, blue, green, and white 7.5 watt light bulbs. The white light was maintained constant at 110 volts. The red, green and blue bulbs were each connected through a 0—110 volt variac so that the voltage to each could be varied through its entire range. Subject was instructed that this was a test to perceive colors, and the range of colors was demonstrated to him. He was instructed to close his eyes and when told to open them to quickly name the color of the filter. The Method of Constant Stimuli was used with red, green and blue colors being presented singly, in random sequence, and with varying voltages. The smallest amount of voltage applied to each lamp where the subject could discriminate the color of the filter from white was taken as the threshold of color detection. The data were evaluated by a Lindquist Type VI Analysis of Variance. No differences attributable to LSD-25 were found.

Our finding of no color detection differences due to LSD-25 is consistent with KRILL's (1960) report. The behavioral effects of the persistently reported b-waves (scotopic) aberrancies (APTER and PFEIFFER, 1957, KRILL et al., 1960) in electroretinographs taken under LSD-25 conditions do not appear to be assessable by this method.

Size constancy. The standard stimulus was a bright yellow isosceles triangle 10 cm. high and 5.5 cm. at the base affixed to a black background one foot square. The variable stimulus was a similar triangle

which was manipulated from 0 to 20 cm. in altitude with a bridle in front of a black background three feet by three feet. The subject was seated 300 cm. from the variable stimulus with the standard stimulus interposed 30 cm. from subject, who was instructed as follows: "The yellow triangle at the end of the room will change in size from large to small or vice versa. When it appears to be the same size as the triangle next to you say 'stop'." The Method of Average Error was used, five ascending and five descending measurements were taken. A task identical to the above except that the standard stimulus was placed 180 cm. from the Subject was also performed. Table 2 comprises the obtained results. A Lindquist Type VI Analysis of Variance was used to evaluate the data.

Mueller-Lyer illusion. The standard stimulus was a black line 13 cm. long with four enclosing lines 3.5 cm. long fixed at 45 degree angles from the end of the line. The variable stimulus extended from the right terminus of the standard, one pair of its expanded end lines being common with the standard, the opposite pair independent. The subject was seated 300 cm. away and instructed as follows: "The variable line will lengthen or shorten. When it appears to be as long as the standard say 'stop'." The Method of Average Error was used, four ascending and four descending trials were administered in random sequence. Table 2 comprises the results. A Lindquist Type I Analysis of Variance was used to evaluate the data.

Table 2. *Spatial perception*

Test	N	Means (cm)		p
		Control	LSD-25	
Size Constancy 10 cm standard				
30 cm.	15	9.89	10.34	<0.01
180 cm.	15	10.26	10.29	<0.70
Mueller-Lyer Illusion 13 cm standard .	15	9.82	9.60	<0.07

Size constancy is the normal, but difficult to understand, tendency for organisms to perceive identical objects at varying distances as the same size. The phenomenon is covered in some detail in WOODWORTH and SCHLOSBERG (1954), but briefly, it can be stated as follows: Where the interocular distance from the lens to the retina is given as d, the size of the image projected on the retina as a, the distance from the lens to an object as D and the size of the object, as A; two similar triangles can be constructed with common apices in the center of the lens. The equation describing the size of the retinal image is then: $a = \frac{Ad}{D}$.

If the size of the retinal image was the appropriate stimulus for object size, then two objects, A_1 and A_2 at distances D_1 and D_2 would

appear the same size when $a_1 = a_2$. Thus, in this experiment, with A_1 (the standard stimulus) = 10 cm., $D_1 = 30$ cm., $D_2 = 300$ cm., A_2 (the variable stimulus) should have appeared the same as A_1 when it was 100 cm. high. Size constancy refers to the phenomenon that, under normal conditions and intermediate distances, A_2 appears the same size as A_1 when they are, in fact, the same size regardless of their differential distances. Size constancy can be nullified by reducing the extra-object visual field with darkness or artificial pupils (HOLWAY and BORING, 1941). To the extent that LSD-25 *functionally* reduces the extra-object visual field, a shift away from constancy, i.e. a tendency requiring the comparison object to be larger than the standard would be expected. This was obtained when the standard was 30 cm. away but not when it was 180 cm. from the subjects.

Although the statistical significance level of the change in the Mueller-Lyer judgments is somewhat indeterminant, the tendency is for subjects to be more susceptible to the illusion under LSD-25. KRUS and WAGNER (1959), using similar procedures did not obtain any effect on the illusion due to LSD-25 but their subjects received only 75 mcg. making direct comparison between the studies difficult.

Warmth detection. Two identical 23.5 watt soldering irons with copper tips 1.5 cm. long and 0.3 cm. in diameter were used. One iron was kept at room temperature, the other iron was connected through a 0—110 volt variac such that its voltage could be varied through the full range. The subject was seated in front of a table and instructed as follows: "Place both of your hands on the table with palms down and close your eyes, I will place one of these irons on the back of each hand. Tell me which is the warmer." The irons were placed on identical contralateral locations, randomly shifted from left to right, and allowed to rest by their own weight. An ascending Method of Limits was used, the voltage being gradually increased on the variable iron between tests until subject responded with four correct identifications. The voltage impressed on the variable iron was taken as the index of heat which could be differentiated from the standard. Table 3 comprises the results, a Lindquist Type I Analysis of Variance was used.

Two-point discrimination. A pair of steel dividers with 21 cm. legs was used, and subject was instructed as follows: "Place your right hand on the table, palm down, and close your eyes; the dividers will be placed on the back of your hand, with either one or two points. Each time this is done say 'one' or 'two'." The spread at which 50% correct responses were received was taken as subject's threshold. Table 3 comprises the results, a Lindquist Type I Analysis of Variance was used.

Cutaneous discrimination, as measured by these indices, is impaired. Neither of these measurements yield absolute threshold data, therefore

Table 3. *Skin sensitivity*

Test	N	Means		p
		Control	LSD-25	
Difference Threshold Warmth .	14	9.18 volt	10.68 volt	<0.09
Two-point Discrimination	5	1.04 cm	2.02 cm	<0.03

direct information about the analgesic properties of LSD-25 cannot be ascertained, only that the ability to discriminate cutaneous sensations is impaired by the drug.

Discussion

No simple generalization is apparent to interpret the data. If processes like attention or discrimination were affected by LSD-25 a differential effect on the reaction time tests could be expected, i.e. the disjunctive or discriminative tests would be more affected by LSD-25 than the simple ones. Instead, a near perfect linearity was obtained; the F-ratio of the drug condition by Reaction Time Test interaction was only 0.28, a result which does not support an "attention impairment" interpretation.

The illusions, i.e., the Mueller-Lyer and Size Constancy were both influenced. Subjects usually reported, although it was not measured here, a pronounced reduction of peripheral vision ("tunneling"). The shift away from size constancy along with the intensification of the M-L effect may both be attributed to a reduction in the visual cues surrounding the stimulus.

The general impairment of skin sensations might lead one to expect a concomitant damping of proprioceptive sensations. Subjects do typically report delusional body sensations, e.g., "My arm doesn't belong to me". If proprioceptive sensations are, in fact, impaired, the nature of the drug effect on the reaction time tasks might be identified.

An overall reduction in sensation, i.e., a damping of somaesthetic information and a reduction of cues from the surround may then account for some of the unusual subjective sensations. The illusogenic effect might represent such a loss of orienting sense data.

Summary

A series of standard tasks: reaction times; simple light, simple buzzer, disjunctive light, and discriminative light; warmth detection, two-point discrimination, size constancy, Mueller-Lyer illusion, and color detection, were administered to subjects under LSD-25 and control states. A general impairment of reaction time and skin sensitivity was obtained. Color detection was unaffected and the illusions were enhanced. The data were interpreted as representing the result of damping of somaesthetic sensations and a reduction in visual cues.

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Psychopharmacologia 2, 304—317 (1961)

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Differential Effects of Phenobarbital, Pentobarbital and Diphenylhydantoin on Motor Cortical and Reticular Thresholds in the Rhesus Monkey* **

By

ROY ASTON and EDWARD F. DOMINO

With 5 Figures in the Text

(Received January 13, 1961)

In 1931, KELLER and FULTON described the action of various drugs on the motor cortex of monkeys. These authors concluded that pentobarbital sodium, in doses which produced light anesthesia, had little effect upon the motor cortex, whereas phenobarbital sodium, administered in equipotent anesthetic doses, abolished motor cortical responses. Subsequently, MERRITT and PUTNAM (1938) found that phenobarbital markedly increased the convulsive threshold for electroshock in cats in non-anesthetic doses, while pentobarbital had little effect except in doses which produced profound drowsiness. Diphenylhydantoin was shown to possess greater anticonvulsant but less soporific effects than phenobarbital. The differential effect of the two barbiturates upon cortical excitability and wakefulness, has been quoted as the basis for the usefulness of phenobarbital in the treatment of grand mal epilepsy (GOODMAN and GILMAN 1955).

The present investigation was undertaken to re-evaluate the previous work of KELLER and FULTON more quantitatively using techniques involving the response of monkeys with chronically implanted electrodes to electrical stimulation of motor cortical and mesencephalic reticular sites.

Methods

Five Macaca mulatta monkeys, of either sex, and weighing from 2 to 4 kg had chronically indwelling stainless steel bipolar electrodes implanted in the mesencephalic reticular formation and, subdurally, on the leg area of motor cortex. Four of these animals also had electrodes placed in the hippocampus. During surgery the animals were anesthetized with pentobarbital. Stereotaxic coordinates for implantation sites

* Supported by grant R-14-59C from the United Cerebral Palsy Research and Educational Foundation, Inc.

** A preliminary report of this study was presented before the American Society for Pharmacology and Experimental Therapeutics Meetings in Chicago (ASTON and DOMINO 1960).

were determined by reference to OLSZEWSKI (1952). The electrodes were fashioned and implanted on the left side according to the method outlined by DOMINO and UEKI (1960). A Cannon plug of 25 contacts was held in a modified "Texas Tower tripod plate. About 300000 units of procaine penicillin were administered to the animals upon completion of the operation and repeated daily for 3 to 4 days. No appreciable infection was noted during a period of 9 months after surgery. All animals were maintained on Purina monkey chow and vitamin supplements.

The implanted areas were stimulated electrically using a Grass stimulator beginning about 1 month after surgery. During the experimental periods the monkeys were isolated in a room separate from the investigator, in which the animals could be observed through a one-way window. The animals were restrained in a "Walter Reed" type chair. The parameters of electrical stimulation were for motor cortex: 60 cps with a pulse width of 1 msec; for reticular formation: 300 cps and a pulse width of 0.5 msec; and for hippocampus: 100 cps with a pulse width of 0.5 msec. In all cases the duration of stimulation was 5 sec. Control thresholds were determined for the three areas under study by stimulating the implantation sites at low voltages and increasing the voltage in steps of 0.2 V until an arbitrarily predefined endpoint was attained. Usually the current strength of stimulation was also determined simultaneously with an oscilloscope. A period of at least 5 min was allowed to elapse between any two successive stimulations.

After obtaining control thresholds various doses of pentobarbital, phenobarbital and diphenylhydantoin, as the sodium salts, were administered intravenously to the animals, and the absolute increase in the electrical threshold for motor, reticular and hippocampal areas was determined. The barbiturates were dissolved in distilled water, and diphenylhydantoin was solubilized in a small volume of 0.1 N sodium hydroxide and subsequently diluted with distilled water to provide a pH of 10.7. Controls for these solvents were included in the study. A single dose of pentobarbital or diphenylhydantoin was injected during one experiment and voltage thresholds determined. The time elapsing between the injection of a drug and the final stimulation in any one experiment varied from 1 to 2 hours with pentobarbital, 5 min being allowed for the onset of drug action, and from 2 to 3 hours with diphenylhydantoin, with 1 hour being allowed for the onset of the drug's effect. Phenobarbital was administered cumulatively with a period of 10 hours between the initial injection and the end of the experiment. After each injection of phenobarbital, 1 hour was allowed for the effect of the drug to develop. In addition to these studies, the dose of barbiturate required to produce loss of both the righting and corneal reflexes in 50% of

monkeys with and without electrode implants was determined as an estimate of the anesthetic AD50. In all cases, at least one week was allowed to elapse between successive drug treatments in any one animal. Doses of drugs employed were 5, 10, 15, 20 and 25 mg/kg of pentobarbital, 40, 60 and 80 mg/kg phenobarbital and 10, 20, 30 and 40 mg/kg for diphenylhydantoin. In the case of the two barbiturates a zero dose refers to control thresholds in the untreated animal. In the case of diphenylhydantoin, the zero dose refers to the response of the monkeys to control injections of solutions of sodium hydroxide adjusted to pH 10.7.

The experimental design used for the study of the two barbiturates was a randomized block, blocks being equated to monkeys. In the case of diphenylhydantoin, a 5 by 5 Latin square design was employed consisting of monkeys, doses and time intervals. Analyses of variance and regression analyses of the data were done according to methods outlined by BURN *et al.* (1950).

All electrode sites were confirmed histologically using the Hess iron deposition technique and thionin counterstaining as modified by DOMINO (1955).

Results

Motor cortex. The endpoint for stimulation of the motor cortex was a flexion, usually of the right leg, followed by minimal clonic activity. Control thresholds over a period of 8 months were very reproducible. The mean threshold $\pm$ SE for 82 observations in the 5 monkeys was 3.2 ± 0.02 V. The effect of intravenous administration of pentobarbital upon the threshold for motor cortical stimulation is illustrated in Fig. 1. In this figure, as in all figures involving responses to barbiturates, each of the five values for control threshold represents a mean of 5 determinations in each monkey, since control thresholds were recorded immediately prior to each injection of pentobarbital. Every other point in this, and succeeding figures, represents a single threshold determination in one animal. Figs. 2 and 3 illustrate the regression lines for phenobarbital and diphenylhydantoin respectively. In all three cases the equation of the line and its approximate 95% limits are included. It is of interest to note that, as calculated by "STUDENT'S" *t*-test, the regression coefficients for these three agents are all significantly different one from another ($P < 0.05$).

When the results of the studies with the two barbiturates were subjected to analysis of variance, it was found that significant differences (< 0.05) occurred between both monkeys and doses, and that for both drugs, the slope of the regression of absolute voltage on dose was significant with no significant deviation from linearity. For diphenylhydantoin, in which the five monkeys were given 5 doses each (including a

sodium hydroxide control) during 5 separate experimental time intervals, it was found, by analysis of variance, that both the animals and the doses, exhibited significant differences among themselves, but that no significant differences occurred among the responses of any one monkey over the 5 experimental intervals ($P<0.05$). In the case of this drug, also, the regression of voltage threshold on dose was not significantly nonlinear.

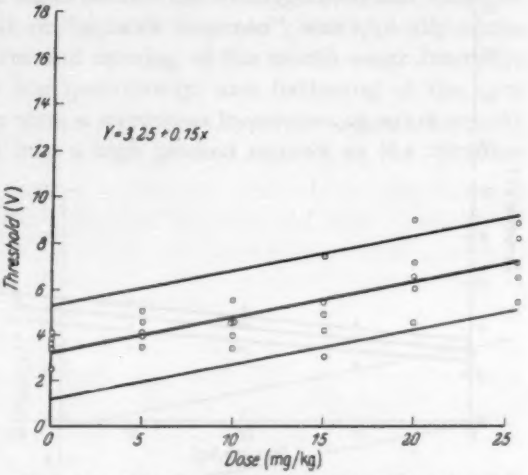

Fig. 1. Regression line and equation of the effect of pentobarbital upon the threshold for electrical stimulation of the motor cortex. In this and all subsequent figures the equation of the regression line of voltage threshold against drug dose in mg/kg given intravenously is shown. The individual points, the regression line and its 95% confidence limits appear as small circles, a thick and two thin lines, respectively

From the regression equations, estimates of the potency of the three agents were calculated as the dose required to elevate the threshold by 50% above the control level. These estimates of "TD50" are 10.8, 19.9 and 35.5 mg/kg for pentobarbital, phenobarbital and diphenylhydantoin, respectively. Expressed as potency ratios, this means that, in terms of the effect in elevating motor cortical thresholds by 50%, pentobarbital was 3.3 times as potent as diphenylhydantoin, while phenobarbital was only 1.8 times as potent. Of the two barbiturates, pentobarbital was 1.8 times as potent as phenobarbital in elevating the motor cortical threshold.

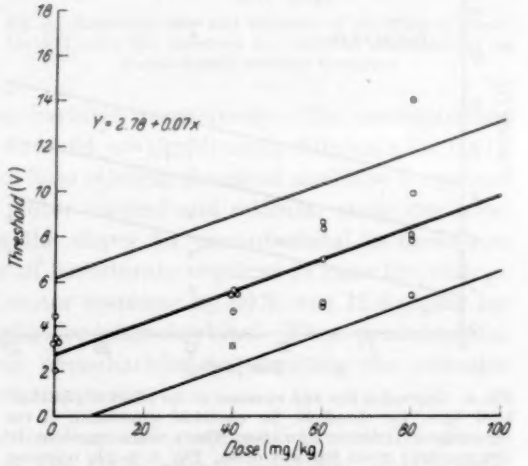

Fig. 2. Regression line and equation of the effect of phenobarbital upon the threshold for electrical stimulation of the motor cortex

The pattern of motor seizures after the administration of each of these agents differed. Although pentobarbital raised the threshold for motor seizures, it had little effect on seizure duration. Before drug treatment in doses of 5 to 20 mg/kg the mean duration was 19.2 sec and after 14.0 sec. This decrease was not significant by the t-test ($P<0.10$). After either phenobarbital or diphenylhydantoin the period of clonus was very much shortened or abolished so that the total period of motor movement at threshold was reduced to the duration of electrical stimulation, i.e. 5 sec. Even increasing the stimulus voltage considerably above threshold failed to prolong the duration of flexion.

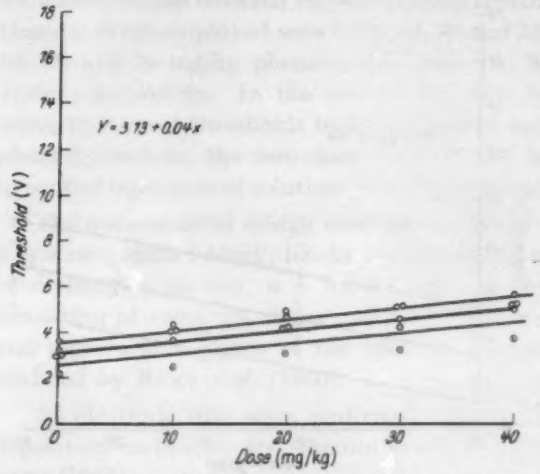

Fig. 3. Regression line and equation of the effect of diphenylhydantoin upon the threshold for electrical stimulation of the motor cortex

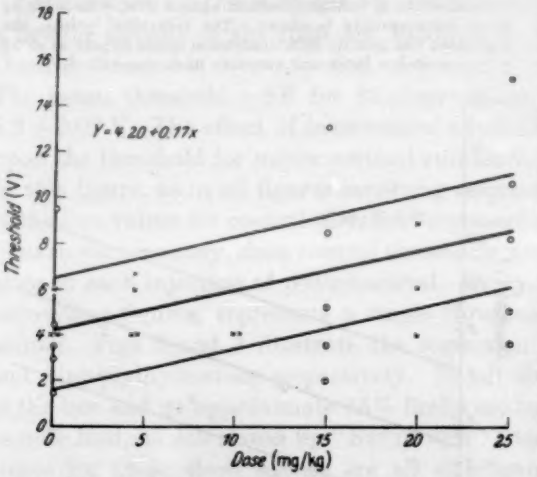

Fig. 4. Regression line and equation of the effect of pentobarbital upon the threshold for electrical stimulation of the mesencephalic reticular formation. The o points represent all five monkeys given different doses. The × points represent the data of various monkeys given intermediate doses

Reticular formation. Two distinct endpoints were chosen for reticular stimulation. No stimulation was given until an animal had lost all behavioral signs of agitation and was sitting quietly. Those animals which had received the larger doses of barbiturates, of course, lapsed into drowsy or sleeping states between stimulations. One endpoint, or "minimal motor response", included behavioral alerting and opening wide of the palpebral fissures, generally together with tightening of the

scalp and wiggling of the ears, slight flexion of one leg or some turning of the head and shoulders to the ipsilateral side. As the voltage was slowly increased the motor signs became more exaggerated and vocalization occurred. This second, or "squawk response", was typically manifested by flexion of the arm and opening of the mouth contralaterally, flexion of the leg at the hip ipsilaterally, and flattening of the ears against the head, together with a stertorous hyperpnea at subthreshold voltages which developed into a high pitched squawk as the stimulus voltage was increased. The mean control threshold $\pm$ SE for 49 observations over 8 months in the 5 animals was 4.9 ± 0.37 V for "minimal motor response" and 6.5 ± 0.25 V for the "squawk response".

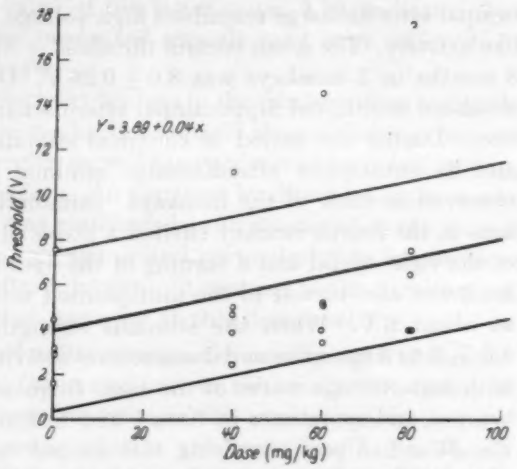

Fig. 5. Regression line and equation of the effect of phenobarbital upon the threshold for electrical stimulation of the mesencephalic reticular formation

The control threshold for the minimal motor response was increased by the two barbiturates employed, but not by diphenylhydantoin. Figs. 4 and 5 illustrate the regression of voltage threshold upon dose for pentobarbital and phenobarbital, respectively. The corresponding regression coefficients, 0.17 and 0.04, are significantly different ($P < 0.01$). The slopes for the regression lines relating threshold and dose for pentobarbital in its effect upon motor cortical and reticular areas are, however, homogeneous, as are the slopes for phenobarbital in these two cases ($P < 0.10$). The doses of barbiturate required to raise the voltage threshold for the reticular motor response by 50% was 12.4 mg/kg for pentobarbital and 48.6 mg/kg for phenobarbital. Thus pentobarbital was 3.6 times as potent as phenobarbital in elevating the reticular threshold for minimal motor response.

Analysis of variance for the "squawk response" to reticular stimulation showed that none of the three agents employed significantly altered the threshold for this phenomenon. Since the voltages required to elicit the "squawk response" were quite high, the phenomenon appeared to be related to supramaximal stimuli and current spread to extrareticular pathways.

Histological examination showed that reticular electrodes were placed at the border between the mesencephalic tectum and tegmentum at the level of the upper border of central grey. Frequently the upper stimulating electrode bordered the superior or inferior colliculus and occasionally was in it.

Anterior hippocampus. The endpoint for stimulation of the hippocampus was taken to be a change in hippocampal EEG activity, which was recorded on an 8-channel ink-writing electroencephalograph. Hippocampal afterdischarge resembled high voltage, hypersynchronized spike-like activity. The mean control threshold $\pm$ SE for 53 observations over 8 months in 3 monkeys was 8.0 ± 0.26 V. Only 3 of the 4 implanted monkeys manifested hippocampal afterdischarge in response to stimulation. During the period of electrical stimulation of the hippocampus and its subsequent afterdischarge minimal gross motor activity was observed in three of the monkeys. Stimulation of the "hippocampal" area in the fourth monkey elicited a ptosis of the right eye with tremor of the right eyelid and a turning of the eyes to the extreme right. The head was also turned to the unimplanted side. This response was seen at about 6 V. When the stimulus strength was increased to about 8.0 V, 3 to 5 cps spike and dome seizure activity was produced in Area 17 with high voltage waves of the same frequency occurring in the hippocampus and sometimes in Area 1 and reticular formation which lasted 25—35 sec. Upon sacrificing this animal one of the "hippocampal" electrodes was found to be located in the lateral geniculate and the other at the superior border of the hippocampus. In the other animals the electrodes were within or bordering the inferior or lateral aspect of the anterior hippocampus.

After all three drugs, the hippocampal threshold showed an elevation in proportion to dose. However, in no case did analysis of variance reveal a significant difference between doses ($P > 0.05$), perhaps due to an insufficient number of trials. In estimating the approximate doses required to elevate the hippocampal threshold by 33%, however, it appeared that phenobarbital was the least potent of the three agents used, while pentobarbital was approximately 3.3 times, and diphenylhydantoin 6.2 times as potent.

Anesthetic effects. The dose of intravenously administered barbiturate required to anesthetize 50% of treated monkeys (AD50) was estimated for pentobarbital and phenobarbital in normal, unimplanted monkeys, and for pentobarbital in implanted monkeys. Five to eight animals were used for each estimate of AD50, this estimate being based on 29 to 30 observations for each treatment. The endpoint chosen as an index of anesthesia was the loss of both righting and corneal reflexes. The results were subjected to probit analysis. In all cases analysis of

variance showed the regression of probit upon log dose to possess a significant slope ($P<0.01$) and to possess no significant deviation from linearity. The table lists the regression equations and the AD50 $\pm$ SE with its 95% confidence limits for each of three treatments. The slopes for the regression lines of the two barbiturates in the unimplanted monkey, 63.64 and 39.46 were not significantly different ($0.50>P>0.10$), as judged by the t-test. Each of these two slopes differs significantly from that of the regression line for pentobarbital in the implanted monkey ($P<0.01$). The low value of the latter slope, 5.19, indicates that anesthetic thresholds in the implanted animals vary more widely than those in unoperated monkeys.

The gross effects of diphenylhydantoin in the monkey were negligible at 10 and 20 mg/kg. At 40 mg/kg the animals showed licking, mucoid salivation, chewing and gagging as well as mydriasis and vertical nystagmus immediately upon injection. An apparent hindlimb muscular weakness also developed which was manifested as an ataxia when the animal was freed in its cage. The nystagmus and particularly the ataxia were evident for several hours after injection. In contrast to the barbiturates, no evidence of deep sedation was seen at this dose level.

Correlation of voltage and milliamperage. Current strength of stimulation was monitored during the major part of this study. However, all threshold determinations were reported in this manuscript in terms of voltage. In order to determine whether this procedure had adversely influenced the data, coefficients of correlation between voltage and milliamperage were determined.

Voltage and milliamperage data were collected for each monkey, from experiments upon both motor and reticular areas over a period of 6 months, providing two estimates of correlation. In 5 monkeys so considered, a total of 10 correlations was determined, ranging from $+0.886$ to $+0.9998$, each of which was statistically significant ($P<0.001$), with a mean of $+0.969$. Also, the extent of correlation between voltage and milliamperage was estimated from 100 observations (20 from each animal) collected over a period of 8 months, with observations representing studies of motor, reticular and hippocampal areas. This provided a correlation of $+0.922$. As might be expected, voltage and milliamperage was much better correlated within one area of one monkey than when all implanted areas were included in the calculation. However, the difference was not statistically significant.

Discussion

Pentobarbital, phenobarbital and diphenylhydantoin were all effective in elevating motor cortical thresholds. Since the regression coefficients for the dose-response curves of these agents were statistically heterogeneous, relative potencies of these agents could not be expressed

as a single figure. If one chose an arbitrary endpoint of a 50% increase in voltage threshold it is evident that pentobarbital was the most potent of the three drugs employed, being 1.8 times as potent as phenobarbital and 3.3 times as potent as diphenylhydantoin. It is of interest that although pentobarbital was the most potent of the three agents studied in elevating motor cortical threshold, it did not appear to alter the duration or the character of the convulsive seizure obtained at threshold voltages. Phenobarbital and diphenylhydantoin in contrast both markedly reduced the clonic component of the induced seizure and shortened its duration. These differences between the two barbiturates together with the finding that the slopes of the regression of voltage threshold on dose differed, suggest that pentobarbital and phenobarbital may increase motor cortical thresholds through different mechanisms.

The observation that phenobarbital raises motor cortical thresholds in monkeys confirms the findings of KELLER and FULTON (1931). The results of the present study on motor cortical thresholds also agree in principle with those obtained by DELGADO and MIHAILOVIĆ (1956) in the monkey, except that, in the latter investigation diphenylhydantoin was found to be more potent in increasing the threshold for motor afterdischarge than phenobarbital. On the other hand, GANGLOFF and MONNIER (1957), working with the rabbit, concluded that diphenylhydantoin affected neither the threshold nor the duration of electrically-induced cortical afterdischarge, and that phenobarbital actually reduced this threshold while having no effect upon duration. Such responses might have been due to very low blood levels of the drugs used since, although these authors administered up to 150 mg/kg of diphenylhydantoin and up to 60 mg/kg of the barbiturate, both agents were given by mouth. In the rabbit, special dietary precautions must be taken to ensure emptying of the stomach (MARKOWITZ 1954), and it is conceivable that the animals employed by GANGLOFF and MONNIER carried undigested food in their alimentary tracts, reducing the rate of absorption of the orally-administered drugs. Furthermore, it has been shown by SCHÜTZ and CASPERS (1953) that with mild depression of the central nervous system, there is an increased tendency to cortical seizures in rats with experimental epileptogenic foci. A low absorption rate of phenobarbital might thus explain the reduced threshold for cortical activation observed by GANGLOFF and MONNIER. It is also well known that low doses of various barbiturates, particularly in rodents such as the mouse, produce marked initial motor stimulation. This is less true of the rabbit. Thus, the former arguments appear less convincing, particularly in view of the fact that GANGLOFF and MONNIER were able to show that phenobarbital in doses lowering motor cortical thresholds markedly elevated diencephalic and rhinencephalic seizure thresholds.

Therefore, species differences may account for the discrepancies observed by various investigators.

The gross behavioral responses evoked by reticular stimulation appear to be similar to those noted by FRENCH (1958) following stimulation of cortical areas projecting into the reticular formation of the monkey. These responses consisted primarily of alerting at low intensities of stimulation and signs of panic at higher intensities. The associated vocalization which was observed in this study was not reported by FRENCH. This is probably due to the fact that the reticular electrodes were high in the mesencephalon and current spread into associated pathways in the superior or inferior colliculi. Both barbiturates raised the threshold for the minimal motor reaction but left the threshold for the "squawk response" unaffected. If phenobarbital is considered a standard, the relative potency of pentobarbital (to cause a 50% increase in reticular threshold) was 3.92. Neither the motor nor the vocal response to reticular stimulation was affected by diphenylhydantoin in the doses used. These data corroborate those of MARTIN, VERNIER and UNNA (1954) who found that phenobarbital depressed the activating response to reticular stimulation while diphenylhydantoin did not. It has been suggested that the reticular formation is one of the principal sites of action of anesthetic agents (FRENCH *et al.* 1953). According to this hypothesis the present results provide some basis for clinical anticonvulsant effectiveness as described previously. Pentobarbital, although the most potent in its ability to increase motor cortical thresholds, would be useless as a chronic medication for the grand mal epileptic because of its marked sedative effects as exemplified by a ratio of motor cortical TD50 to reticular TD50 of only 1.15. Phenobarbital, on the other hand, manifests a two-fold increase in this ratio of 2.44. Diphenylhydantoin, although the least potent of the compounds studied in terms of motor cortical depressant effects nevertheless had no demonstrable action upon reticular thresholds and therefore showed the greatest ratio. The determination of the anesthetic potencies of the two barbiturates provided the same order of potency as did the studies on reticular thresholds. Pentobarbital was 6.24 times as potent as phenobarbital. In the doses employed, diphenylhydantoin showed no general anesthetic properties. It is obvious that effective elevation of motor cortical thresholds can be accomplished without markedly altering the reactivity of the reticular core to electrical stimulation or producing significant anesthesia. These results help explain the observation of MERRITT and PUTTMAN (1938) that diphenylhydantoin increases electroshock thresholds without producing marked sedative effects. Thus the present investigation provides further scientific rationale for the clinical usefulness of phenobarbital and diphenylhydantoin in grand mal and

Table. *Anesthetic dose regression lines for pentobarbital and phenobarbital given intravenously*

Drug	No. of observations	Regression line	AD 50 and mg/kg	± S. E.	Per cent error	95% Limits
Pentobarbital (implanted monkey)	29	$Y = -0.65 + 5.19 \times$	12.26	1.91	15.58	9.03— 16.65
Pentobarbital (unimplanted monkey)	30	$Y = -71.69 + 63.64 \times$	16.33	0.31	1.90	16.02— 16.65
Phenobarbital (unimplanted monkey) . . .	30	$Y = -74.21 + 39.46 \times$	101.74	1.11	1.09	99.61—103.92

cortical focal (Jacksonian) epilepsy. However, it should be pointed out that the pyramidal system is not crucial to the development of generalized seizures (GASTAUT and FISHER-WILLIAMS 1959). Thus electrical stimulation of the motor cortex may in no way mimic the site of origin of grand mal epilepsy. In fact there is evidence of many important subcortical mechanisms in such seizures. Nevertheless, electrical stimulation of the motor cortex is a very convenient means of initiating abnormal seizure discharge in the experimental animal.

In the present study all three drugs employed showed a tendency toward elevation of the voltage threshold for electrical afterdischarge in the hippocampus. Diphenylhydantoin appeared to be the most potent and phenobarbital the least potent. Both barbiturates were less effective in raising the hippocampal threshold than in raising the cortical threshold. Diphenylhydantoin proved to be more potent in its hippocampal than in its motor cortical effects. The preliminary findings of an elevation in hippocampal thresholds by phenobarbital in monkeys confirms the work of GANGLOFF and MONNIER (1957) in rabbits. These authors, however, found no effect of diphenylhydantoin on rhinencephalic seizure thresholds. Species differences of inadequate absorption of diphenylhydantoin administered orally might account for this negative finding as described previously.

The results of the anesthetic studies with barbiturates indicate that, in those monkeys which had implanted cerebral electrodes, the variation in response to single doses of the agent was greater than that in unoperated monkeys. This is evident if the standard error of the AD50 is expressed as a percentage of the AD50. If this is done the "% error" in the unimplanted monkeys is 1.09 and 1.90% for phenobarbital and pentobarbital respectively, while that for pentobarbital in implanted animals is 15.58% (see table). Simultaneously solving the regression equations for pentobarbital in the implanted and unimplanted monkeys gives

the intersection of these two lines at a point corresponding to 16.44 mg/kg and 50.5% response. At doses below 16.44 mg/kg the implanted animals are more susceptible to the effects of the drug, while at doses above 16.44 mg/kg the implanted animals are more resistant to the drug's effects than the unimplanted ones. The results obtained in this study using low doses are in keeping with the results of SEQUIN and STAVRAKY (1957), HELLER *et al.* (1960), and ADLER (1960) who found that brain lesions including the septal forebrain, frontal cortex or caudate nucleus markedly increase barbiturate sleeping time. It is possible that the anterior hippocampal implantations in the monkeys used in the present study may have had an influence upon septal activity since these two areas are known to possess fibre connections.

The extent of activation of the neuronal mass at the electrode tip is dependent upon the energy supplied by electrical stimulation. Energy imparted by electrical circuits is directly proportional to the current strength, duration of application and tissue impedance. Since the latter may vary, milliamperage appears to be the parameter of choice for studies of electrical threshold. Both amperage and voltage have been used in previous studies. In the present investigation a significant correlation has been shown to exist between milliamperage and voltage threshold determinations. Thus, if the regression of milliamperage on some variable is linear, the relationship between this variable and voltage will also manifest linearity. In this study the quantification of drug effect upon threshold of stimulation is dependent solely upon the establishment of linear dose-response relationships and, for this reason estimates of voltage threshold were considered adequate.

Summary

Rhesus monkeys were implanted chronically with bipolar electrodes in motor cortical, mesencephalic reticular and anterior hippocampal areas. Voltage and current thresholds of stimulation were determined for the elicitation of gross motor seizures, alerting responses, and electrical seizures, respectively before and after varying doses of pentobarbital, phenobarbital and diphenylhydantoin. Anesthetic potencies were also estimated. Diphenylhydantoin was the most specific in terms of increasing motor cortical thresholds in doses that had no significant effect upon reticular thresholds. Phenobarbital was somewhat less specific than diphenylhydantoin. It elevated motor cortical thresholds in doses which only minimally increased reticular thresholds. Pentobarbital increased both motor cortical and reticular thresholds.

The potency in elevating motor cortical thresholds was inversely related to the degree of specificity. Thus, pentobarbital was the most

potent, phenobarbital second, and diphenylhydantoin least effective in increasing motor cortical thresholds. In elevating the threshold for hippocampal afterdischarge phenobarbital appeared to be the least potent while diphenylhydantoin appeared the most potent.

Anesthetic potencies of these drugs followed the same trend as did their effects on reticular thresholds. Thus, pentobarbital was the most potent, phenobarbital second and diphenylhydantoin was least effective having no anesthetic effects.

Voltage thresholds were found to be significantly correlated with milliamperage thresholds.

It is concluded that this study provides additional scientific rationale for the clinical usefulness of phenobarbital and diphenylhydantoin in the treatment of cortical focal (Jacksonian) and grand mal epilepsy.

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A New Technique for the Investigation of some Analgesic Drugs on a Reflexive Behavior in the Rat

By

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With 2 Figures in the Text

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A number of attempt have been made to develop a method of measuring in animals the effects of clinically established analgesic drugs which would adequately reflect the important aspects of their analgetic effects in man (BEECHER 1957). In this connection, a procedure developed by KIMBLE (1955) appears promising. He has demonstrated that an electric shock delivered to a rat's feet through the grid floor of a testing apparatus will elicit two distinguishable responses: at low shock values the animal will "crouch" or "flinch" while at higher shock values the animal will "jump".

There are several advantages to this procedure, as opposed to some of the previously used techniques (HILL et al. 1957; COOK and WEIDLEY 1957; ERCOLI and LEWIS 1945). First, since prior training is unnecessary, it is unlikely that the drug effects demonstrated by the performance of the animal would be due to drug actions on recent memory or other variables associated with training. Second, the elicitation of two discrete responses to different intensities of the same noxious stimulus offers the advantage of considering, by analogy, the relationship of these responses to the responses of human subjects to painful stimuli (HARDY, WOLFF and GOODELL 1952). If the "flinch" response, produced at low shock levels, reflects the first perception of pain by the animal and the "jump" response, produced at higher shock levels, reflects an emotionally influenced reaction of the animal to the pain, then the present procedure provides measures of the same factors which have been proposed as important in the human response to pain.

Method

The experimental animals were 156 male hooded rats of the Long-Evans strain. The animals were between 100 and 250 days old.

The testing apparatus consisted of a $8 \times 14''$ box with a grid floor and a clear plastic front. An electric shock of controllable amperage at approximately 230 volts was delivered through a commutator to the grid floor from a Model 228 Applegate electronic stimulator. This stimulator provides a constant current source which prevents changes

in skin resistence to affect current density. The shock durations and the intervals between shocks were timed by electronic timers.

The same testing procedure was used with all animals. Each rat was placed in the testing apparatus and a series of unavoidable shocks were delivered to the animal's feet. Each animal was administered 14 series of electric shocks. The different shocks intensities within a series were presented according to a method of limits procedure. Each series consisted of 10 stimulations at the following shock intensities in ma: 0.1, 0.2, 0.3, 0.4, 0.9, 1.4, 1.9, 2.4, 2.9, 3.4. The shocks were presented in an ascending or descending order on alternate series, with the first series for each animal being an ascending one. Each shock was of 1.0 sec duration. The shocks were presented at 30.0 sec intervals. The time interval between each series of shocks was two minutes.

After each shock one of the following three responses was recorded: no response, "flinch", or "jump". "Flinch" was defined as a "startle" or "crouch" behavior in which the animal's feet did not leave the grid. This response was seldom accompanied by any signs of agitation. The "jump" response was defined as a removal of two or more paws from the grid at the time of the shock onset. This response appeared to be a highly emotional reaction which was usually accompanied by vocalization, running, and other signs of agitation.

All animals were injected intraperitoneally with isotonic saline solutions of the drugs. No more than 0.8 ml was injected for any drug solution. The average volume for most of the drugs was 0.2 ml. Forty control animals, some of which were later used in the drug groups, were injected with 0.2 ml isotonic saline. Twenty of these saline control animals were tested one hour after injection and 20 were tested two hours after injection to control for effects of the injection-test interval. Two different injection-test intervals were necessary to ensure measurement at approximately peak action of the drugs tested.

Table 1 presents the drugs and doses tested. Four different dose levels were tested for each drug except for the combinations of amphetamine-codeine and morphine-nalorphine. Only one dose level was employed for these mixtures. For all agents except reserpine each animal was tested at all dose levels of a drug. The order of dose administration was counterbalanced with a minimum of 48 hours between tests. Six animals were tested on each drug except reserpine. Animals receiving amphetamine, B-phenylisopropylhydrazine (PIH), B-phenylisobutylhydrazine (Lakeside's JB 835), iproniazid, codeine, morphine, amphetamine-codeine, nalorphine nalorphine-morphine and sodium acetylsalicylate were tested one hour after drug administration. Those receiving chloropromazine, perphenazine, tetrabenazine, and reserpine were tested two hours after drug administration.

The observers in this experiment were aware of the names of the drugs being administered but were unaware of the expected action. Data sheets were taken from the observers after each rat's performance so they could not check back on previous scorings.

Table. *Doses of Drugs Tested*

Drug	Doses tested in mg/kg			
Sodium acetylsalicylate	100	250	500	750
Codeine sulfate	30	50	70	90
Morphine tartrate	2	3	4	5
Nalorphine hydrochloride	0.5	1.0	1.5	2.0
Chlorpromazine hydrochloride	2	4	6	8
Perphenazine dihydrochloride	0.2	0.3	0.4	0.5
Iproniazid phosphate	10	20	30	40
β-phenylisopropylhydrazine (PIH)	3	4	5	6
β-phenylisobutylhydrazine (JB 835)	2	3	4	5
Dextroamphetamine sulfate	2.5	3.0	3.5	4.0
Tetrabenazine monosulfate	4	6	8	10
Reserpine (each dose given on one of four consecutive days)	0.3, 0.3, 0.7, 0.7	0.5, 0.5, 1.0, 1.0	0.7, 0.7, 1.2, 1.2	1.0, 1.0, 2.0, 2.0
Mixture of morphine tartrate and nalorphine hydrochloride	0.5 nalorphine 5.0 morphine			
Mixture of codeine sulfate and dextroamphetamine sulfate	2.5 d-amphetamine 30.0 codeine			

The 24 animals receiving reserpine were tested two hours after the last of four injections. Each animal was injected once each day for four consecutive days. Due to the cumulative effects of this drug, six different animals were tested at each of the four dose schedules. For example, Table shows that one group received 0.3 mg/kg on the first two days and 0.7 mg/kg on the third and fourth days.

Results

The threshold for each response, i.e., "flinch" or "jump", was defined as the shock value at which the animal responded on 13 of the 14 series. Thus, if an animal "jumped" on 13 out of the 14 series at 1.50 ma, this would be its "jump" threshold. The threshold was calculated by interpolation from the animal's response to the shock intensities tested, i.e., the psychophysical method of limits procedure.

For control animals injected with isotonic saline the mean value at which the threshold for the "flinch" response occurred was 0.20 ma with a SD of 0.08. The mean threshold for the "jump" response was 1.04 ma with a SD of 0.10. Inter observer reliability coefficients exceeded $r = 0.95$. This demonstrates that the response categories are readily

identifiable. No difference in either threshold was found between the one and two hour post-injection testing times. A split-half reliability of the test between the first seven and the last seven trials for the saline condition yielded an $r = 0.87$.

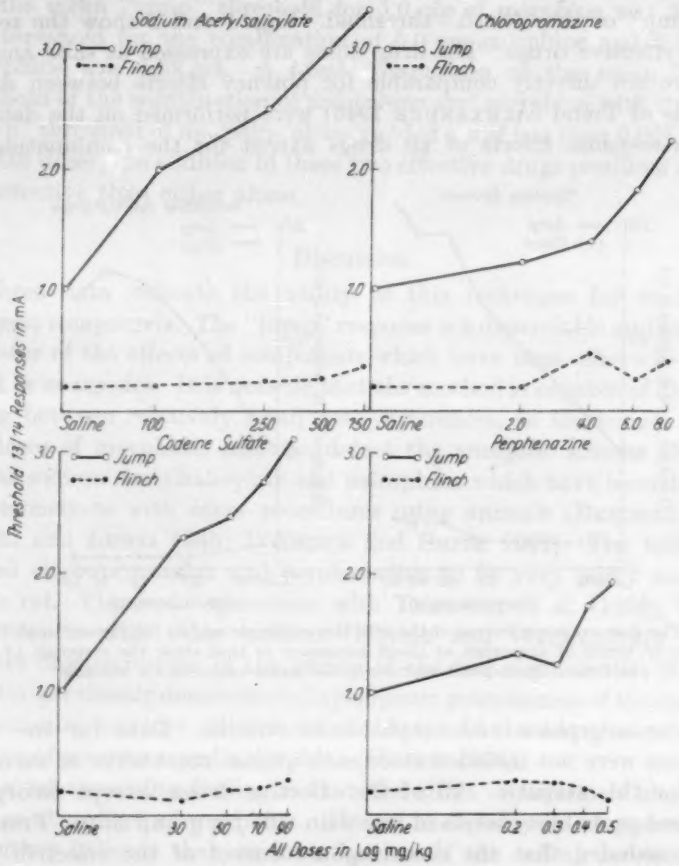

Fig. 1. The dose-response curves of the effective analgesic agents for "jump" and "flinch" responses in terms of amperage of shock necessary to first elicit the response 13 out of 14 times. Each point represents the mean value for six animals

Even at the highest dose tested none of the drugs completely abolished either the "flinch" or the "jump" response at the higher shock values. No qualitative changes in the responses were noted at even the highest doses tested; this suggests that a motor deficit was not interfering with the performance of the responses when a sufficient shock was delivered to ensure elicitation of the response.

None of the drugs produced a significant change in the "flinch" threshold. Sodium acetylsalicylate, codeine, morphine, nalorphine, per-

phenazine, and chloropromazine produced changes in the "jump" threshold, and in each instance, except nalorphine, obvious dose-response relationships were obtained. Iproniazid, PIH, JB 835, reserpine, tetrabenazine, and amphetamine produced no demonstrable changes in either the "jump" or the "flinch" threshold. Figs. 1 and 2 show the results for the effective drugs. The drug doses are expressed as salts and are therefore not directly comparable for potency effects between drugs.

Tests of Trend (ALEXANDER 1946) were performed on the data for all dose-response effects of all drugs except for the combinations of

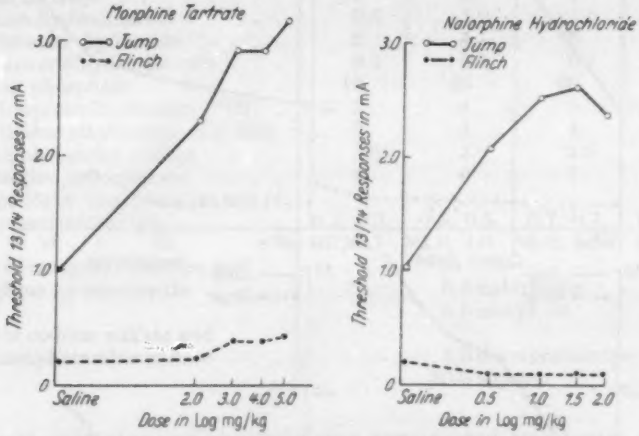

Fig. 2. The dose-response curves of the effective analgesic agents for "jump" and "flinch" responses in terms of amperage of shock necessary to first elicit the response 13 out of 14 times. Each point represents the mean value for six animals

morphine-nalorphine and amphetamine-codeine. Data for the saline condition were not included since each animal must serve at each dose level for this statistic. All of the effective drugs, except nalorphine, produced probability levels of less than 0.05 for group slope. From this it is concluded that the dose-response curves of the effective drugs deviated significantly from the horizontal. The doses of nalorphine tested were apparently too large to yield a dose-response effect. However, a comparison of mean "jump" threshold of the lowest value obtained for nalorphine with saline shows nalorphine to be an effective compound ($p < 0.001$).

The mean "jump" threshold for 2.5 mg of amphetamine was 1.37 ma. This value was not significantly different from the mean "jump" threshold for saline. The mean "jump" threshold for 30.0 mg of codeine was 2.28 ma. The mean "jump" threshold for the combination of 2.5 mg amphetamine plus 30.0 mg codeine was 2.88 ma. A t-test comparison of the mean "jump" threshold of codeine alone with the codeine plus amphet-

amine combination yielded a p of less than 0.05. Thus, at these doses, the addition of amphetamine, which itself is not effective, increases the activity of codeine.

The mean "jump" threshold for nalorphine at 0.5 mg was 2.09 ma, and the mean "jump" threshold for 5.0 mg of morphine was 3.18 ma. The threshold for the combination of 5.0 mg morphine and 0.5 mg of nalorphine was 1.58 ma. A t-test comparison of the mean "jump" threshold of the combination of nalorphine and morphine with the mean "jump" threshold of morphine alone yielded a p of less than 0.001. Thus, at these doses, the addition of these two effective drugs produces a result less effective than either alone.

Discussion

These data indicate the utility of this technique for evaluating analgesic compounds. The "jump" response is a dependable and sensitive indicator of the effects of compounds which have been otherwise established as analgesics. It is notable that the method is capable of discriminating between relatively small dose differences, as illustrated by the low doses of morphine, and can detect the analgetic actions of drugs such as sodium acetylsalicylate and nalorphine which have been difficult to demonstrate with other procedures using animals (Beecher 1957; Ercoli and Lewis 1945; D'Amour and Smith 1941). The technique showed chloropromazine and perphenazine to be very midly analgetic in the rat. This is in agreement with Tedeschi et al. (1959), but in disagreement with other authors (Houde and Wallenstein 1955). Another demonstration of the utility of the technique is that it could show the previously demonstrated synergistic potentiation of the codeine-amphetamine mixture (Goetzl et al. 1944) and the antagonism of the mixture of morphine and nalorphine (Woods 1956).

A most surprising finding is that the effect of aspirin is almost as great as the effect of morphine. This is unexplainable at present but the author believes that an investigation of the ceiling levels of the drugs will be greatly different. To support this is the consideration of the very low dose of morphine being used in this study. An investigation of ceiling effects and of potency comparisons of standard opiates is now being performed.

It might be that the sensitivity of the method in detecting the analgetic effects of established clinical analgesics is due to the utilization of a response which is influenced by the emotional reaction of the animal to the pain. Beecher (1958) has proposed that most or all of the analgetic action of a drug is derived from its effect on the emotional reaction to pain rather than on the animal's absolute threshold of pain perception. The finding that the "jump" response was affected by known analgesics

and that the "flinch" was not seems to be consistent with this hypothesis. The emotional behavior of vocalization and agitation associated with the "jump" response also lends support to this interpretation of the Beecher hypothesis. Whether the "flinch" response indicates the first perception of pain by the animal or merely the perception of an electric shock is impossible to determine.

The results obtained from testing the psychotropic drugs suggests that brain concentrations of norepinephrine and serotonin are not related to analgetic action. The two phenothiazine "tranquilizers", which do not affect the concentration of these brain amines (JACOBSEN 1960) appeared to be analgesics by this technique. Reserpine and tetrabenazine, both of which have been shown to deplete the brain of norepinephrine and serotonin (BRODIE et al. 1959) were shown to be non-analgetic. Finally, PHI, JB 835, and iproniazid, the monoamine oxidase inhibitors, which protect these brain amines from destruction (BRODIE et al. 1959) were also shown to be non-analgesics.

Summary

The analgetic action of a series of analgesic and psychotropic agents was tested in a situation in which variable intensities of electric shock to a rat's feet were used to elicit two distinguishable reflexive responses: a "flinching" response at low shock values, and a "jumping" response at higher shock values.

By using a modified method of limits, reliable threshold for the two responses were obtained.

Chlorpromazine, perphenazine, morphine, codeine, nalorphine and acetylsalicylate were found to raise the threshold to "jump", but had little or no effect on the threshold to "flinch".

PIH, JB 835, iproniazid, reserpine, tetrabenazine, and amphetamine were found to have no effect on either the "jump" or the "flinch" thresholds.

A combination of amphetamine and codeine was found to produce a synergistic potentiation. A combination of morphine and nalorphine was found to produce an antagonism.

The results were discussed in terms of BEECHER's hypothesis that the analgetic action of drugs is due to a diminution of the emotional components of an animal's reaction to pain and in terms of the relationship of brain amine change to analgetic action.

Acknowledgement. The drugs for this study were obtained from the following companies: Lakeside Laboratories, Schering Corp., Smith, Kline, and French, and Hoffman-LaRoche Inc. Technical assistents for the study were HOWARD L. SETSER, RONALD W. MORTON and WILLIAM BRADLEY.

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The Effect of Monoamine Oxidase Inhibitors on the Deconditioning Action of Reserpine in Rats

By

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With 4 Figures in the Text

(Received April 17, 1961)

Pretreatment with inhibitors of monoamine oxidase has been shown by various authors to antagonize many of the effects of reserpine including the sedation, miosis, palpebral ptosis, the cardiovascular effects and the toxicity of the alkaloid (CHESSIN *et al.*; BESENDORF and PLETSCHER) as well as its effects on the EEG (SAVOLDI *et al.*).

Investigation in the field of psychopharmacology has demonstrated both in the rat and in the monkey, that reserpine is capable of depressing either a conditioned response or an operative response (GHUA *et al.*; WEISKRANTZ and WILSON; BRADY). Therefore, it was decided to examine the character and the degree of this general antagonism exhibited by the MAO inhibitors in relation to the characteristic deconditioning effect of reserpine. Accordingly, a study was made of the relationship between some MAO inhibitors and reserpine in rats which had been conditioned to an escape reaction, in this case, the "barrier-crossing response". The apparatus used was derived from the Warner cage and consists of a cage with a metal grill floor, one half of which could be energized with an electrical current alternately and automatically with the other thereby compelling the animal to pass from one side of the compartement to the other avoid a shock.

Materials and methods

Male albino rats of the Wistar type weighing 240—350 g were used. The automatic device employed was that described recently by BOVET *et al.* and is illustrated in Fig 1. The tests were carried out daily using a simple programme of a duration of 25 minutes which corresponded to 50 stimuli. The unconditioned stimulus (U.S.) consisted of an electrical shock of 65 V. The conditioning stimulus (C. S.) was a light of variable duration which remained lit until the animal responded with either an unconditioned (U.R.) or the conditioned escape reaction (C. R.). Before beginning the treatment, the animals were subjected to repeated trials until they attained a high level of conditioning (more than 80% of the responses were conditioned responses).

Three monoamine oxidase inhibitors of different chemical structure were studied: nialamid [N-benzyl-β-(isonicotinylhydrazine)-propionamide]; pheniprazine (β-phenyl-isopropyl hydrazine, Catron) and I.S. 2596 (N′-(1,4-benzodioxan-2-methyl)-N′-benzylhydrazine). The latter was recently described by BOVET-NITTI *et al.*

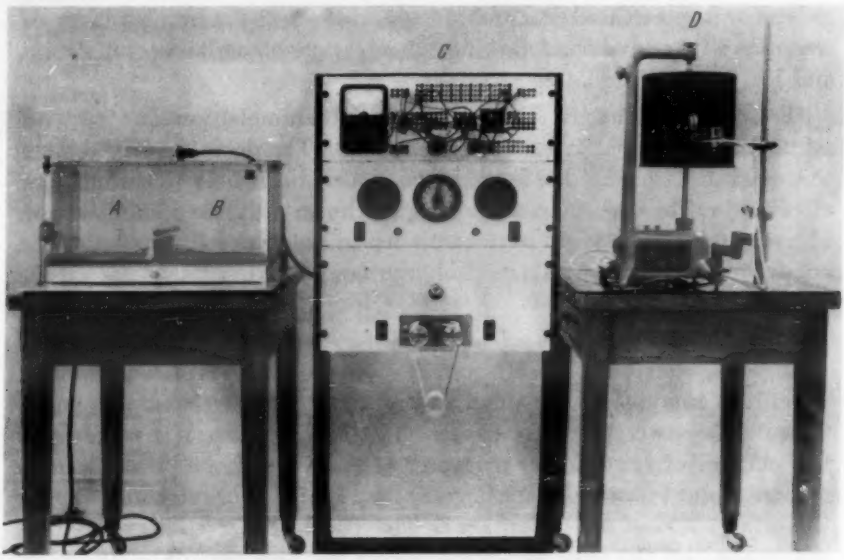

Fig. 1. Apparatus for the study of the "barrier-crossing response" in the rat

A and *B*, the two compartement cage; above the cage, a white light of an intensity of 3 candlepower provided the conditioned stimulus; the metal grill floor of the cage was charged with a shock of 65 V a.c. as the unconditioned stimulus.

C, electronic programmer with continous moving programme tape, which allows an independent variation of the total duration of the experiment, the duration of the cycle, the quality, intensity and duration of the conditioned stimuli, the duration and intensity of the unconditioned stimuli and the latency between conditioned and unconditioned stimuli.

D, recording device consisting of one electromagnetic inscriber recording all changes of compartement by the animal and a second inscriber recording only those changes in compartement between the beginning the conditioned stimulus and the beginning of the unconditioned stimulus, i.e. the conditioned reactions.

The experimental programme used consisted in daily trials of 50 cycles of 30 sec each. A cycle was as follow:

1. At 0 sec., i.e., the beginning of the cycle, the floor grids of compartements *A* and *B* were uncharged. The lamp (C.S.) in the ceiling of the cage became energized and remained lit until the animal in compartement *A* crossed to compartement *B*.

2. At 5 sec., the current (U.S.) was switched on in the grid of compartement *A* for 25 sec., i.e., until the end of the first cycle. It could not be avoided by the animal unless it passed from compartment *A* to compartment *B*, i.e., until the rat sought refuge in the other half of the cage.

3. At 30 sec. a new cycle began.

Two schedules of treatment with reserpine were used: in schedule I, a mild and repeated dosage, the animals were given 0.5 mg/kg s.c. each day for three successive days; in schedule II the more severe treatment, a single injection of 1 mg/kg s.c. was given.

The group receiving the inhibitors consisted of 5 rats each. Employing reserpine, schedule I, each of 3 groups was pretreated, 4 hours before reserpine, with one of the following compounds: pheniprazine, 5 mg/kg i.p. on the first day and 2.5 mg/kg i.p. on each successive day; nialamid and I.S. 2596, 10 mg/kg i.p. each day. With treatment schedule II, in which the single dose of reserpine was given each of three additional groups was pretreated 24 and 4 hours before the administration of reserpine with one of the following: pheniprazine, 5 mg/kg i.p.; nialamid and I.S. 2596, 10 mg/kg i.p.

For each experiment, a control group (10 animals) was treated with saline and the respective doses of reserpine. The doses employed were well tolerated and no deaths occurred either during the experiment or afterwards. The experiments were begun 2 hours after the administration of reserpine and the trials were continued in the days following until the conditioned response in the control group recovered.

Results

Effect of reserpine. The effect of reserpine on the rats of conditioning, when it is administered in small and repeated doses (schedule I), manifests itself in two successive pnases. In the first, there is a decrease in the number of conditioned response, a retention of the unconditioned response, and to a significant degree, the capability of retraining during the brief period of the experiment. This can be observed on the curves determined by the C.R. occurring during the five periods of 10 cycles making up the test. These curves are of the ascending type, normally observed in untrained animals, and demonstrate the progress of learning during the experiment (Fig. 2, control, days 3 and 4). In a second phase, there is a greater and successive diminution in the frequency of the conditioned response which falls to zero, a decrease in the unconditioned response and a complete loss of the ability to relearn during the trial such that instead of an ascending learning curve, a descending curve which can be interpreted as a fatigue reaction, is now seen (Fig. 2, control, days 5 and 6). These results are also clear when the analysis is made with respect to the individual rats (Fig. 3).

Following the administration of the higher dose of 1 mg/kg, schedule II, the second phase, seen with lower dose schedule, appears directly, the animals showing a pronounced decrease in the conditioned response and a decrease in the unconditioned response as well. The deconditioning reached a maximum in 24 hours and an effect is still evident at 48 and 72 hours after the administration of the reserpine (Fig. 4, control).

It is interesting to note that, in the animals deconditioned by the reserpine treatment, a high level of conditioning reappears as the effect

of reserpine wears off without the intervention of additional trials. This can be seen quite clearly in Fig. 2, control, where after the sixth day, there was an interval of two days without trials and where it is evident that the marked increase in performance on the ninth day constitutes a return to practically control levels of conditioning rather than a relearning process.

Interaction between reserpine and MAO inhibitors. Pretreatment with pheniprazine or I.S. 2596 provides, with reserpine schedule I, an effective

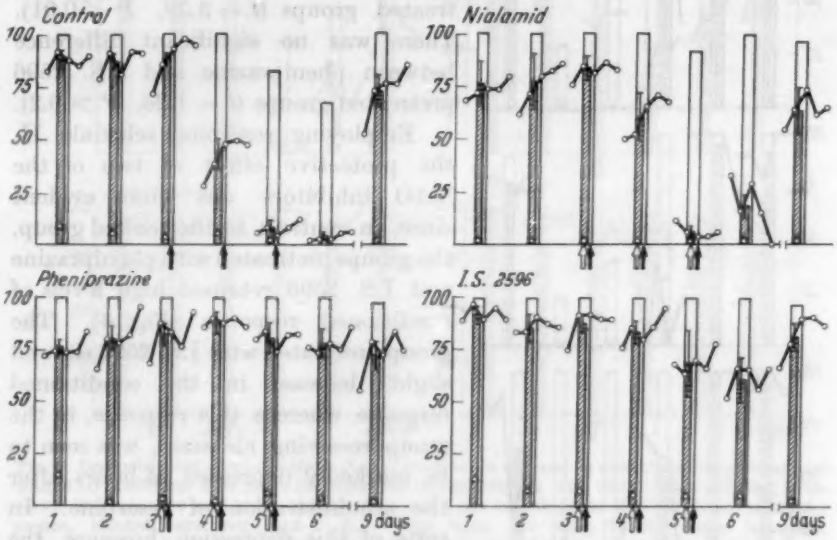

Fig. 2. Deconditioning effect of small and repeated doses of reserpine in rats and its antagonism by pretreatment with MAO inhibitors. This figure illustrates the results obtained with daily trials (50 cycles, 30 sec. each). No trials were made on days 7 and 8. Ten rats were used in the control group and 5 rats in each of the MAO inhibitor pretreatment groups. Shaded bars, per cent C.R.; white bars, per cent U.R.; white triangles, percentage of cycles in which there occurred one or more "additional responses"; open circle of black line, percentage of C.R. occurring during the five periods of 10 cycles making up the test; vertical lines at the top of the bars, standard errors of the C.R. White arrows, injection of MAO inhibitors 4 hours before reserpine treatment: nialamid, 10 mg/kg i.p.; pheniprazine, 5, 2.5, 2.5 mg/kg i.p.; I.S. 2596, 10 mg/kg i.p. Black arrows, reserpine 0.5 mg/kg s.c.

protection against the deconditioning action of reserpine which is seen experimentally as a maintenance of the high percentage of conditioned responses and the typical ascending learning curves (Fig. 2). The protective effect of nialamid was appreciably less, the animals exhibiting a marked fall in conditioned response after the third dose of reserpine. Moreover, learning curves of the descending type are seen indicating a decraesed capacity to "relearn", the animals at this point appearing to be in a fatigue-like state. However, it should be noted that the level of unconditioned response was maintained to a higher degree than of the control in which there was seen a marked decrease in both the conditioned and the unconditioned response.

A t-test carried out on the results obtained on the sixth day showed a significant difference for each pretreated group from the control (nialamid, $t = 3.70$, $P < 0.01$; pheniprazine, $t = 13.23$, $P < 0.001$; I.S. 2596; $t = 6.95$, $P < 0.001$). There was a significant difference between the pheniprazine pretreated group and the nialamid pretreated group ($t = 6.95$, $P < 0.001$) and between I.S. 2596 and nialamid pretreated groups ($t = 3.39$, $P < 0.01$). There was no significant difference between pheniprazine and I.S. 2596 pretreated groups ($t = 1.26$, $P > 0.2$).

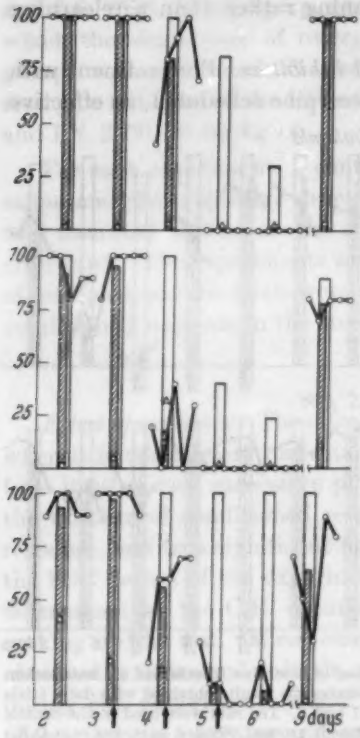

Fig. 8. Analysis of the behavior of three individual control rats receiving reserpine treatment schedule I. At arrows, reserpine 0.5 mg/kg s.c. No trials were made on days 7 and 8. After the interval there is seen, in all three rats an abrupt return to high levels of conditioning from a state of practically complete deconditioning. Note in the lower chart the lack of effect of reserpine on U.R. in contrast to the marked effect on the C.R.

Employing reserpine schedule II, the protective effect of two of the MAO inhibitors was more evident since, in contrast to the control group, the groups pretreated with pheniprazine and I.S. 2596 retained high levels of conditioned response (Fig. 4). The group pretreated with I.S. 2596 showed slight decrease in the conditioned response whereas this response, in the group receiving nialamid, was seen to be markedly depressed 24 hours after the administration of reserpine. In spite of this depression, however, the capacity to relearn, indicated by ascending learning curves, remained unaltered (Fig. 4). On the other hand, pheniprazine provided a complete protection against the deconditioning effect of reserpine.

Statistical treatment of the results obtained on the fourth day shows a significant difference for each pretreated group from the control (nialamid, $t = 3{,}65$, $P < 0.01$; pheniprazine, $t = 35{,}1$, $P < 0.001$; I.S. 2596, $t = 11{,}16$, $P < 0.001$). There was a significant difference between the pheniprazine pretreated group and the nialamid pretreated group ($t = 3.16$, $P < 0.02$). There was no significant difference between I.S. 2596 and nialamid pretreated groups ($t = 2.04$, $P > 0.05$) and between pheniprazine and I.S. 2596 pretreated groups ($t = 1{,}58$, $P > 0.1$).

The "additional responses", i.e., the crossing of the animal without being provoked by either the optical or electrical stimulus, which can be considered a measure of the state of anxiety, remained at the same level during the entire experiment both in the control groups and the treated groups.

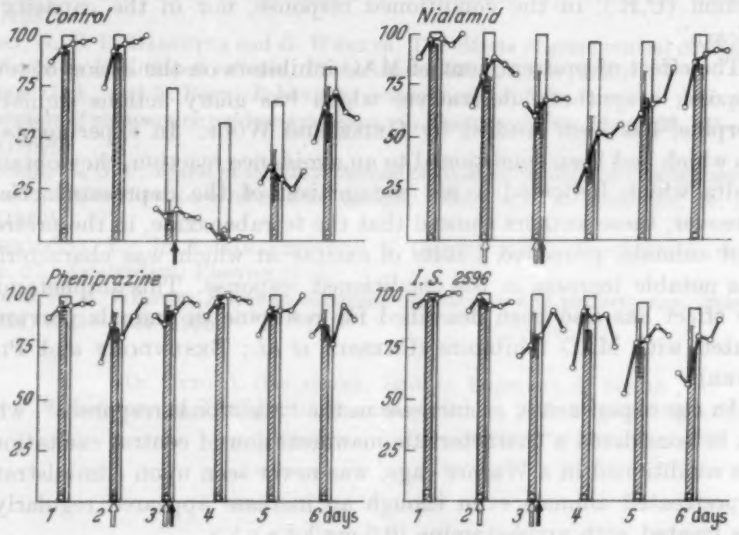

Fig. 4. Deconditioning effect of a single dose of reserpine in rats and its antagonism by pretreatment with MAO inhibitors. This figure illustrates the results obtained with daily trials (50 cycles, 30 sec each). Ten rats were used in the control group and 5 rats in each of the MAO inhibitors pretreated groups. Shaded bars, per cent C. R.; white bars, per cent U. R.; white triangles, percentage of cycles in which there occurred one or more "additional responses", open circles of black line, percentage of C.R. occurring during the five periods of 10 cycles making up the test; vertical lines at the top of the bars, standard errors of the C.R. White arrows, injection of MAO inhibitors 24 and 4 hours before reserpine treatment: nialamid, 10 mg/kg e i.p.; pheniprazine 5 mg/kg i.p.; I.S. 2596, 10 mg/kg i.p. Black arrows, reserpine 1 mg/kg s.c.

Discussion

The results of the experiments with reserpine alone confirm, in a general sense, the observation by various authors of the depressant action of reserpine on conditional behavior (GUHA *et al.*; WEISKRANTZ and WILSON; BRADY). However, it was shown that whereas reserpine depressed performance, it had no effect on retention. WEISKRANTZ in experiments on monkeys, demonstrated that with doses of reserpine sufficiently high, the animals were incapable of even the slightest degree of conditioning; notwithstanding, the present results show clearly that the animals do not lose a previously acquired conditioning.

The antagonism to the action of reserpine by the MAO inhibitors was manifested on various aspects of the behavior of the rats. Under the experimental conditions described, a complete protection from the effect

of reserpine was observed with pheniprazine; a significant effect was seen with I.S. 2596 and a less intense effect was obtained with nialamid. In particular, it was shown that reserpine had no sedative effect in these rats previously treated with pheniprazine or I.S. 2596; the animals seemed quite normal exhibiting no modification of the escape motor reaction (U.R.), in the conditioned response, nor in the capacity to "relearn".

The effect of pretreatment of MAO inhibitors on the action of tetrabenazine, a synthetic derivatives which has many actions similar to reserpine, has been studied by HEISE and WOLF. In experiments on rats which had been conditioned to an avoidance reaction, they obtained results which indicated a net antagonism of the depressant effects. Moreover, these authors showed that the tetrabenazine, in the pretreatment animals, provoked a state of excitation which was characterized by a notable increase in the conditioned response. This amphetamine-like effect has also been described for reserpine in animals previously treated with MAO inhibitors (CHESSIN *et al.*; BESENDORF and PLETSCHER).

In our experiments, an increase in the "additional responses", which can be considered a characteristic manifestation of central excitation in rats conditioned in a Warner cage, was never seen upon administration to pretreated animals even though an increase appeared regularly in rats treated with amphetamine (0.5 mg/kg s.c.).

Summary

The effects of two different dosage schedules of reserpine and pretreatment with inhibitors of monoamine oxidase were studied on rats conditioned to the "barrier-crossing response" in the Warner cage.

Reserpine provoked a gradual deconditioning when it was administered in small and repeated doses; with a single high dose, the deconditioning was abrupt.

Pheniprazine, nialamid and I.S. 2596 (N'-(1,4-ben odioxan-2-methyl)-N'-benzylhydrazine) were capable of preventing the deconditioning effect of reserpine.

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Die Wirkung des Chlorpromazins auf den Kohlenhydratstoffwechsel des Rattengehirns

Von
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Biochemische Untersuchungen mit Phenothiazinen ergeben, daß die Zellatmung durch diese Pharmaka charakteristisch beeinflußt bzw. gehemmt wird [Berti u. Mitarb. 1953; Peruzzo u. Mitarb. 1953; Courvoisier u. Mitarb. 1956; Abood u. Mitarb. 1955; Andrejew u. Mitarb. 1956; Bernsohn u. Mitarb. 1956; Century u. Mitarb. 1956; Decsi 1956; Hadnagy u. Mitarb. 1958 (u.a.)].

Beim Studium des Wirkungsmechanismus von Arzneimitteln, die in die Aktivität des Gesamtorganismus tief eingreifen, ist es wichtig, nicht nur die Änderungen der oxydativen Prozesse, sondern auch jene des Kohlenhydratstoffwechsels zu untersuchen. Das trifft besonders für das Gehirn zu, wo die Kohlenhydrate die wichtigste Rolle für den normalen Hirnstoffwechsel spielen. Im Vergleich zu den sich mit den Oxydationen befassenden Arbeiten ist die Zahl derjenigen, die sich mit den Änderungen des Kohlenhydratstoffwechsels beschäftigt, relativ gering (Decsi 1956; Bernsohn u. Mitarb. 1956). Deshalb haben wir es uns zur Aufgabe gemacht, in akuten und chronischen Versuchen die Wirkungen des Chlorpromazins auf den Gehalt des Hirns an gesamtreduzierenden Stoffen und Glykogen zu untersuchen.

Methodik

Die Untersuchungen wurden an 83 männlichen und weiblichen, sexuell nicht reifen Wistar-Ratten, im Gewicht von 30—50 g durchgeführt. Sämtlichen Tieren wurde dieselbe gemischte Diät (Zerealien, Speiseabfälle, Grünfutter) verabreicht. Fünfzehn Tiere (7 männlich, 8 weiblich) dienten für akute Versuche. Die intraperitoneal eingespritzte Chlorpromazin- [2 Chlor (3-dimethylamino-3′-propyl) 10-phenothiazin, Largactil, Firma Specia, Paris] Dosis betrug 0,35 mg/100 g Körpergewicht. Auf Grund von Vorversuchen mit jeweils um 0,05 mg pro 100 g steigenden Dosierungen von 0,10—0,70 mg/100 g wurde die Dosis von 0,35 mg/100 g gewählt, weil dies die kleinste Dosis war, die ohne Ausnahme jene Veränderungen hervorrief, die bei der Ratte unter Einwirkung von Phenothiazinen vom Typus Chlorpromazin als charakteristisch

gelten. Denn wir beabsichtigten, die biochemischen Veränderungen in der Phase des ausgeprägten Hemmungszustandes zu untersuchen, der durch Chlorpromazin hervorgerufen wird. Den Kontroll-Tieren (14 männliche, 9 weibliche) wurde intraperitonell dasselbe Volumen physiologischer Kochsalzlösung injiziert.

Die chronischen Versuche wurden mit 30 Tieren begonnen. Die täglich einmal, subcutan injizierte Chlorpromazin-Dosis betrug 0,10 mg je 100 g. Mit den chronischen Versuchen sollte gewissermaßen ein „Modellversuch" in Analogie zur Humantherapie durchgeführt werden. Die obige Menge entspricht etwa der mittleren menschlichen therapeutischen Dosis. Die Behandlung dauerte für 15 Tiere (7 männliche, 8 weibliche) 2 Wochen und für die übrigen 15 (6 männliche, 7 weibliche) 4 Wochen. Wir verloren in der letzten Gruppe 2 Tiere; das eine wegen eines Unfalls, das andere verendete an einer interkurrenten Krankheit. Für die chronischen Versuche dienten 17 Tiere (9 männliche, 8 weibliche) als Kontrollen. Diesen Ratten wurde täglich einmal subcutan physiologische Kochsalzlösung injiziert.

Bei den akuten Versuchen wurden die Tiere 35 min nach der Einspritzung des Chlorpromazins in toto in flüssige Luft gelegt; bei der chronischen Behandlung 24 Std nach der letzten Injektion. Vor dem Versuch wurde die Kopfhaut der Ratten auf dem Scheitel enthaart, die Haut wurde mit Tusche bezeichnet. Das Einfrieren dauerte 3 min. Dann wurde der Kopf mittels eines Meißels vom Körper getrennt und verblieb bis zur Verarbeitung in flüssiger Luft. Bei der weiteren Verarbeitung wurde der Kopf mit einem Hammerschlag auf den Meißel in zwei Hälften gespaltet. Nachdem die Gewebe des Kopfes einigermaßen aufgetaut waren, hoben wir das Gehirn aus dem Schädelraum. Die Gehirnhälften wurden mit flüssiger Luft wieder eingefroren und in gefrorenem Zustand in einem holzumkleideten, geschlossenen Waagegefäß auf einer Analysenwaage gewogen. Dann wurden die Hemisphären in Eprouvetten des Homogenators von Potter-Elvehjem, in 4 ml 90%iges Äthanol verbracht. Diese Alkohol-Konzentration wurde gewählt, weil sie von Kerr zum Fällen des Glykogens empfohlen wird und die im Gehirn befindliche Glukose in 90%igem Äthanol löslich ist. Das Homogenat wurde nach 20 min Stehen bei Zimmertemperatur 30 min mit einer Tourenzahl von 4800/min zentrifugiert. Der Überstand wurde abgegossen, 1 ml davon wurde mit $CuSO_4$—Na_2WO_4 nach Somogyi enteiweißt (1 ml Überstand, 0,6 ml 7% $CuSO_4$ cryst., 0,18 ml Aqua dest., 0,6 ml 10% Na_2WO_4). Nach 10 min Stehen des Gemisches wurde erneut mit derselben Tourenzahl 20 min zentrifugiert. Die gesamtreduzierenden Substanzen wurden in 1 ml dieses Überstandes nach Somogyi-Nelson (1944) analysiert. Wir bestimmten das Glykogen in dem Rückstand des Alkoholhomogenats nach Kerr-Szára-Bagdy (1936, 1953). Die

Reproduzierbarkeit der beiden von uns angewandten Methoden wurde vor den Hauptversuchen durch entsprechende Standard- und Standard-Gehirn-Versuche mehrfach kontrolliert.

Ergebnisse

Änderungen des Verhaltens. Während des *akuten Versuchs* beobachteten wir deutliche Beeinflussung des allgemeinen Verhaltens der Tiere. Die Ratten wurden unbeweglich, verloren den Orientierungsreflex. Die Körperhaltungsreaktionen blieben erhalten, doch neigten die Tiere dazu, paradoxe Körperlagen zu fixieren: wenn wir sie z.B. am Rande des Tisches hängen ließen, blieben sie angeklammert, ohne wieder hinaufzuklettern oder herabzufallen. Diese Symptome begannen 10—15 min nach der Injektion, fingen 40—60 min nach der Einspritzung an abzuklingen und gingen nach einer Dauer von 3—3½ Std zu Ende.

Während der *chronischen Behandlung* waren wir genötigt, den Anfangs- und Endpunkt des Hemmungszustandes an einer Gruppe von jeweils 15 Tieren festzustellen. Die Ratten, die einer Versuchsgruppe angehörten, wurden nämlich in einem Käfig gehalten.

Festgehalten wurden: 1. die Latenzzeit, d.h. die Zeit von der Injektion bis zum Auftreten von deutlichen Hemmungserscheinungen bei der Mehrzahl der Tiere; 2. die Hemmungsdauer oder die Zeit vom Eintritt bis zum Zeitpunkt des Verschwindens der Hemmung bei der Mehrzahl der Tiere. Da diese Bestimmungen nur durch Beobachtung — stets durch dieselbe Person — gemacht wurden, haben sie nur relative und nicht quantitative Bedeutung, was deshalb genügt, weil sie nur ein Bild über den Verlauf des Verhaltens der chronisch mit Chlorpromazin behandelten Tiere geben wollen.

Die Einzeldosis der chronischen Behandlung rief eine wesentlich weniger ausgeprägte Hemmungswirkung hervor als die ungefähr dreifache Dosis im akuten Versuch, der Orientierungsreflex blieb normal oder wurde für kurze Zeit gegen Ende der zweiten Woche sogar gesteigert. Auch die Körperhaltungsreaktionen blieben bei der Mehrzahl der Tiere unverändert; die Neigung zur Fixierung paradoxer Körperlagen fehlte.

Bei der chronischen Behandlung war der Ablauf der Symptome wie folgt: In der ersten Woche begann die Hemmung des allgemeinen Verhaltens ungefähr 70 min nach der Injektion und dauerte ungefähr 50 min. Gegen Ende der ersten Woche war das Verhalten der Ratten jenem morphinbehandelter Ratten ähnlich: sie waren munter, lebhaft, und wälzten sich herum. Dieses Verhalten verschwand nach einigen Tagen. Zwischen dem 7.—10. Tage betrug die Latenzzeit 110 min, die Hemmung dauerte ungefähr 40 min. Zwischen dem 10.—14. Tage war sowohl die Latenz als auch die Hemmungsperiode ungefähr 40 min. Während der zweiten Woche waren die Tiere auch außer der Zeit der unmittelbaren Arzneimittelwirkung durch einen Hemmungszustand charakterisiert. Sie waren den ganzen Tag hindurch bewegungsärmer als die Kontrolltiere. Brachten wir sie aber in eine andere Umgebung, z. B. in einen anderen Käfig, liefen sie mit hastigen raschen Bewegungen hin und her, viel heftiger als die Kontrollen; bald sanken sie dann aber in den

geschilderten Hemmungszustand zurück. Sie waren besonders gegen Schallreize empfindlich. Diese Schallempfindlichkeit verschwand nach einigen Tagen. In der dritten Woche dauerte die Latenz 35 min, die Hemmungsperiode 40 min. In der vierten Woche dauerten Latenz- und Hemmungsperioden gleicherweise 25 min. Nach dem Verlauf der zweiten Woche außer in der Periode der unmittelbaren Arzneimittelwirkung unterschieden sich die Ratten in ihrem Verhalten nicht mehr von den Kontrollen. Wir weisen aber darauf hin, daß die Registrierung der Hemmung durch einfache Beobachtung nicht ohne weiteres die Abwesenheit feinster Spuren der Hemmung bedeutet.

Biochemische Daten. Tabelle 1 enthält die Mittelwerte ($\bar{x}$) und die Standardabweichungen (s) der gesamtreduzierenden Stoffe und des Glykogengehaltes bei den Kontrollen, nach einer einmaligen Chlorpromazin-Injektion und nach zwei- bzw. vierwöchiger chronischer Behandlung.

Tabelle 1. *Die Wirkung einer einmaligen Chlorpromazin-Dosis und einer chronischen Chlorpromazin-Behandlung auf den Gehalt an gesamtreduzierenden Stoffen und Glykogen des Rattengehirns* (mg/100 g Feuchtgewicht). *Mittel und Standardabweichung*

$n=40$				$n=15$				$n=15$				$n=13$			
a) Kontrollen				b) Akuter Versuch				c) Chronischer Versuch (zweiwöchige Behandlung)				d) Chronischer Versuch (vierwöchige Behandlung)			
Gesamtreduzierende Stoffe		Glykogen		Gesamtreduzierende Stoffe		Glykogen		Gesamtreduzierende Stoffe		Glykogen		Gesamtreduzierende Stoffe		Glykogen	
$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
28,3	4,86	59,4	10,99	34,1	5,99	61,1	14,83	41,2	4,70	62,3	10,26	36,0	8,12	68,7	8,44

In dem akuten Versuch fanden wir bei 23 Kontrolltieren die folgenden Mittelwerte ($\bar{x}$) und Standardabweichungen (s): Für die gesamtreduzierenden Substanzen $\bar{x}=28{,}8$, $s=5{,}89$ und für den Gehalt an Glykogen $\bar{x}=60{,}7$, $s=15{,}21$. In den chronischen Versuchen bei 17 Kontrollratten waren die Werte für Gesamtreduktionsstoffe $\bar{x}=27{,}6$, $s=3{,}02$ und für den Glykogengehalt $\bar{x}=57{,}7$, $s=7{,}26$. Bei der Auswertung der Ergebnisse vereinigten wir die zwei Kontrollgruppen, und in Tabelle 1 wurden die vereinigten Werte angegeben. Wir halten dieses Verfahren für zulässig, da kein wesentlicher Unterschied zwischen den entsprechenden Mittelwerten der zwei Kontrollgruppen besteht. Die Vereinigung beider Gruppen hat deshalb keine Änderung der statistischen Ergebnisse zur Folge.

In Tabelle 2 sind die Ergebnisse der statistischen Auswertung aufgeführt (Formel nach STUDENT), wobei $\bar{x}=$ der Unterschied der Mittelwerte der verglichenen Gruppen, $s=$ die gemeinsame Standardabweichung beider verglichenen Gruppen, $t=t$-Wert der Formel von STUDENT, $P=$ der Wahrscheinlichkeitsgrad; unter „Signifikanz" ist die Qualifikation der statistischen Rechnungen ausgewiesen.

Tabelle 2. *Die Mittelwerte der gesamtreduzierenden Stoffe und des Glykogengehaltes im Rattengehirn bei akuter, zweiwöchiger und vierwöchiger Chlorpromazinbehandlung im Vergleich zu den Kontrolltieren und in Abhängigkeit von der Dauer der Behandlung*

	a		b		c		d	
	Gesamt-reduzierende Stoffe	Glykogen	Gesamt-reduzierende Stoffe	Glykogen	Gesamt-reduzierende Stoffe	Glykogen	Gesamt reduzierende Stoffe	Glykogen
$\bar{x}$	5,8	1,7	12,9	2,9	7,7	9,3	5,2	6,4
s	5,19	12,3	4,82	10,81	5,80	8,70	6,51	9,46
t	3,69	0,46	8,84	0,89	4,16	3,55	2,11	1,79
P	$<0{,}01$	$>0{,}60$	$<0{,}01$	$>0{,}20$	$<0{,}01$	$<0{,}01$	$<0{,}05$	$<0{,}10$
Signifikanz	ja	nein	ja	nein	ja	ja	ja	nahe

a) Der Unterschied der Mittelwerte der Gesamtreduktionsstoffe und des Glykogengehaltes nach einer einmaligen Chlorpromazin-Injektion im Vergleich zu den Kontroll-Mittelwerten.

b) Der Unterschied der Mittelwerte beider Metabolite nach zweiwöchiger Chlorpromazin-Behandlung im Vergleich zu den Mittelwerten bei den Kontrollen.

c) Der Unterschied der Mittelwerte beider Metabolite nach vierwöchiger Chlorpromazin-Behandlung im Vergleich zu den Kontroll-Mittelwerten.

d) Der Unterschied der Werte der 2 bzw. 4 Wochen lang behandelten Tiere.

Aus Tabelle 1 und 2 ist folgendes ersichtlich:

1. Die einmalige Chlorpromazin-Dosis erhöhte den Gehalt des Gehirns an gesamtreduzierenden Stoffen. Der Unterschied zu den Werten bei den Kontrollen ist statistisch signifikant. Der Glykogengehalt blieb unverändert.

2. Nach zweiwöchiger Behandlung fanden wir ebenfalls eine signifikante Zunahme der gesamtreduzierenden Stoffe. Der Glykogengehalt blieb auch zu dieser Zeit unverändert.

3. Nach vierwöchiger Behandlung waren sowohl die Gesamtreduktionsstoffe wie auch die Glykogenwerte signifikant höher als bei den Kontrollen.

4. Nach vierwöchiger Behandlung waren die Gesamtreduktionswerte signifikant niedriger als bei den 2 Wochen lang behandelten Tieren. Der Glykogengehalt nahm gleichzeitig nicht signifikant zu.

Diskussion

Kulenkampff (1951) wies in einer histochemischen Arbeit nach, daß seine Resultate dann auswertbar wurden, wenn er seine Tiere aus möglichst großen Würfen auswählte und diese sexuell nicht reif waren. Unsere eigenen Versuchstiere wählten wir nach der Erfahrung dieses Verfassers aus.

In unseren akuten Chlorpromazin-Versuchen haben die Gesamtreduktionswerte im Rattengehirn zugenommen. Dafür möchten wir eine

Störung im Verbrauch der gesamtreduzierenden Substanzen verantwortlich machen. Im Hirnhomogenat bilden die Glucose und ihre reduzierenden Spaltprodukte die sog. gesamtreduzierenden Stoffe. Die reduzierenden Aminosäuren kommen hier nicht in Betracht. STRANGE u. Mitarb. (1955) wiesen nämlich nach, daß nur Tyrosin, Tryptophan und Cystein eine Reduktionsfähigkeit besitzen und in Hirnhomogenaten der Maus fanden ROBERTS und FRANKEL (1950) mit einer papierchromatographischen Methode keine der erwähnten drei Aminosäuren.

Die Ergebnisse der chronischen Chlorpromazin-Behandlung bedürfen einer besonderen Besprechung. Wie wir sahen, reagiert der Organismus auf das Chlorpromazin in den verschiedenen Perioden der Behandlung unterschiedlich: Das Verhalten der Tiere schien bis zur Mitte der Behandlung zunehmend gehemmt, später ließ die Hemmung graduell nach. Auch andere Stoffwechselprozesse verändern sich im Laufe einer chronischen Behandlung. FILK u. Mitarb. (1954) beobachteten z.B. einen verminderten Sauerstoffverbrauch am Rattengehirn nach Chlorpromazin-Gaben. Wenn sie es jedoch wiederholt verabreichten, blieb der vermindernde Effekt trotz Steigerung der Dosis aus. Wir fanden die höchsten Gesamtreduktionswerte nach zweiwöchiger Zufuhr, d.h. in jenem Zeitpunkt, als das allgemeine Verhalten der Tiere am stärksten gehemmt war. Nach vierwöchiger Behandlung waren die Reduktionswerte zwar noch erhöht, aber signifikant niedriger als nach zweiwöchiger Behandlung. Wir halten es für möglich, daß die Erhöhung der Gesamtreduktionswerte im Gehirn der Tiere eine gewisse Art Hemmung zu erzeugen vermag.

Die cerebrale Glykogenkonzentration nahm in unseren Versuchen zur gleichen Zeit deutlich zu, in der die Reduktionswerte abnahmen, d.h. sozusagen auf deren Kosten. Der Zusammenhang der eben geschilderten Prozesse ist noch unbekannt, insbesondere, weil die Aktivitätsänderungen der Fermente, die für die Zucker-Polymerisation verantwortlich sein könnten, noch nicht untersucht sind.

Im Gegensatz zu früheren Befunden, kamen mehrere Verfasser zu dem Ergebnis, daß sich der cerebrale Glykogengehalt unter variablen experimentellen Bedingungen ändert (PALLADIN 1956; MÁTHÉ u. Mitarb. 1959). Diese Ergebnisse können dafür sprechen, daß das Glykogen im Gegensatz zu der früheren Auffassung doch aktiv am Stoffwechsel teilnimmt. PROHOROVA (1954) brachte dafür einen direkten Beweis. Sie untersuchte die Austauschgeschwindigkeit der C^{14}-Glucose sowohl im Glykogen der Leber wie auch im Gehirn und fand sie in beiden Organen gleich groß; in manchen Fällen übertrafen sogar die Werte des Gehirns jene der Leber. Es erhebt sich daher die Frage, ob der erhöhte cerebrale Glykogengehalt während der Chlorpromazin-Behandlung möglicherweise einen vorteilhafteren Energiezustand für das Gehirn bedeutet.

Summary

The effect of chlorpromazine [2-chlor (3-dimethylamino-3′-propyl) 10-phenothiazin] on the glycogen content and the level of the reducing substances has been studied in the rat brain during acute and chronic experiments.

The single dose of 0,35 mg/100 g chlorpromazine given intraperitoneally elevates the level of the reducing substances significantly, while the glycogen content remains unchanged.

After the treatment for two weeks with the dosage of 0,10 mg/100 g per day results a significant increase in the level of the reducing substances and an unchanged quantity of glycogen.

After the treatment for 4 weeks, the level of the reducing substances decreases significantly; at the same time the glycogen content raises above the control value significantly.

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Control for Stimulus-Change in the Evaluation of Alcohol and Chlorpromazine as Fear-Reducing Drugs*

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With 2 Figures in the Text

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As Miller (1957) has pointed out, virtually all studies designed to investigate differential effects of drugs on fear, or aversion, have used an unbalanced design which seriously limits any conclusions that may legitimately be drawn from them. Since all animals are trained under a non-drug condition, subsequent placebo tests are *similar* to the conditions of original training, while subsequent drug tests are *different* from these conditions. This design is illustrated in the top half (I) of Table 1.

Table 1. *Balanced design to control for effects of stimulus-change*

Training	Testing
I. Placebo	A. Placebo (similar) B. Drug (different)
II. Drug	C. Placebo (different) D. Drug (similar)

What are the effects which might be expected from this unbalanced design? We know that some drugs make the subject feel different and/or change his perception of the external environment. For example, alcohol can produce a variety of bodily sensations and cause the environment to seem to move unsteadily. Such drug-produced stimulus changes will be expected to cause a decrement in the strength of habits which were originally learned under the non-drug condition and then generalized to the new drugged condition.

Furthermore, Murray und Miller (1952) and Miller and Kraeling (1952) have shown that the strength of avoidance responses, motivated by fear, is reduced more by a stimulus change than is the strength of approach responses, motivated by hunger. For example, the latter authors trained hungry rats to run down an alley to get food and then gave them electric shocks at the goal until they stopped running down to it. Half of the rats were tested in the same alley and still stopped running; the other half were tested in a somewhat different alley (changed in brightness and width) and resumed running. But this tendency to resume running is exactly the same effect that is produced by certain drugs, such as alcohol (Conger 1951). Heistad (1958) has performed

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an experiment which indicates that the same type of analysis may be employed to explain the effects of electroconvulsive shocks and chlorpromazine.

Since we know that stimulus change can produce the same behavioral effects that certain drugs produce in a conflict situation, how can we test to see whether the drug effect is a mere by-product of stimulus change, or a genuine effect on the strength of the physiological mechanism of fear? The way to do this is to use the neglected half of the completely balanced design. The procedure is to train animals under a drug, and then test half of them under the *same* drug condition, while the other half is tested under a different non-drug condition. This is illustrated in the bottom half (II) of Table 1.

If the drug produces its effects primarily as a by-product of stimulus change, we will expect the animals tested under the same drugged condition to be relatively unaffected, while those tested under the different placebo condition should be the ones to show the greatest reduction in fear and resume the performance that achieves the goal of food. Thus the results of the two halves (I and II of Table 1) of the balanced experimental design should be exactly *opposite*. The theoretical implication of such a result is that the drug produces its effects primarily by changing the stimulus situation; the practical implication is that we may expect the drug to have opposite effects under different circumstances.

On the other hand, if the drug produces its effect primarily by acting directly on the physiological mechanism for fear, we will expect it to produce the same kind of effects in both halves of the experimental design and we will be safer in generalizing these results to a greater variety of practical conditions.

The main purpose of the present study was to use this completely balanced design in evaluating the possible fear-reducing effects of two commonly used drugs: alcohol and chlorpromazine. Since we were evaluating each of these drugs separately, rather than comparing them, we used single doses of each drug at what appeared to be an effective level on the basis of the literature and exploratory work in our laboratory.

In order to obtain a true measure of the drug effects on fear, uncomplicated by possible analgesic effects on the pain which is presumably induced by electric shock, each *S* was tested without electric shock (fear test) as well as with it (fear-plus-pain test) under both drug and placebo conditions.

Method

Subjects and feeding schedule. Twenty-four naive male albino rats of the Sprague-Dawley strain (Holzman Co., Madison, Wisconsin), 90 to 100 days old at the beginning of the experiment, were used. Throughout

the study, *S*s were maintained on a restricted feeding schedule allowing 12 g of dry food (Purina Laboratory Checkers), in addition to 1 g (average) of wet mash, obtained in two daily test sessions. This feeding schedule resulted in a mean weight loss of 28 g over a four-week test period.

Apparatus. A variable-length "telescope" alley (MILLER and BARRY 1960) allowing repeated measures of performance in an approach-avoidance conflict situation, was employed. The $4 \times 5 \times 72$ in. straight-alley had black wooden walls, a transparent Plexiglas top, and a grid floor made of parallel stainless steel bars, $^1/_{16}$ in. in diameter, placed $^3/_8$ in. apart. A 7 in. startbox was separated from the rest of the alley by a guillotine door. The opposite end of the alley was formed by a galvanized-steel panel, covered by clear Plexiglas, which could be inserted at 1-foot intervalls, 1–6 feet from the startbox.

A stainless-steel food cup, $^1/_2$ in. in diameter and $^3/_{16}$ in. deep, was mounted on the center of the movable panel so that the cup projected 1 in. into the end of the alley, $^3/_4$ in. above the grid floor.

An electric timer started automatically when the door of the startbox was raised. Speed of running was recorded by means of photoelectric cells placed at 1-foot intervals throughout the alley. When *S* made contact between the grid and the food cup, an electronic relay stopped the last timer and, on appropriate trials, delivered an electric shock to the grid. This shock was delivered from a voltage divider through a 100000 ohm resistor in series with the grid, for a duration of 0.2 sec.

Procedure. *Approach-training.* All *S*s received four days of preliminary training as follows: On the first day, the animals were allowed to wander about on a table top where small pellets of wet mash were randomly placed. On the second day, six spaced trials were given in the alley, one at each alley length, which increased from 1–6 feet in 1-foot intervals on successive trials. *S*s were returned to their home cage for 10 min between trials.

On days three and four, 12 massed trials were given per day, *S*s running the sequence from 1–6 feet twice in succession without additional temporal spacing between the two test sessions. On the basis of average performance (running speed) on the fourth day, the animals were divided into four equated groups of six *S*s each. Grups I and I′, serving as controls for this phase of the experiment, were trained under a placebo (isotonic saline) condition. Grups II and II′ received the subsequent avoidance training under alcohol or chlorpromazine respectively.

Throughout the experiment, *S*s were retained in the startbox for a minimum of 5 sec, and the door of the alley was raised only when the animal faced in the direction of the door. The reward consisted of one pellet of wet mash, weighing approximately 0.1 g. If *S* failed to touch

the food cup within 30 sec during any one of the trials of the first test session, the animal was immediately returned to the startbox and started on the second session. If *S* failed to touch the food cup within the required time during the second session of each day, the test was terminated and the animal returned to the home cage.

Dosages. For the alcohol condition, intraperitoneal injections of 15 cm^3/kg of a 10% alcohol solution were given, 10 min before the first test session of each day. A matched group of animals received placebo injections of isotonic saline of comparable volume also 10 min before the experiment.

For the chlorpromazine condition, intraperitoneal injections of 2 mg per kg of chlorpromazine in a 1 cm^3/kg solution of isotonic saline were given one hour before the first session. A matched group of animals received placebo injections of isotonic saline of comparable volume also one hour before the experiment.

Avoidance training with and without drugs. Throughout the avoidance training as well as subsequent drug and control tests, two test sessions of six trials each were run per day. During the first two days of avoidance training both sessions were identical. No shock was given in the 1-foot alley on the first trial of each session. Each subsequent trial was run to a distance greater by 1 foot than the preceding trial, the increment in length being correlated with an increase in shock-intensity. The shock voltage was increased by 30 volts for each added foot of alley length, from 60 volts in the 2-foot alley to 180 volts in the 6-foot alley.

In order to separate drug effects which are specific to the pain which is presumably induced by the electric shock (i.e. purely analgesic effects), from effects that are specific to the cues signalling shock (i.e. fear-reducing effects), two test sessions were given. The first (fear only) session of each day had electric shock only at the end of the last trial run to the farthest distance, so that behavior could not be influenced by any analgesic effects of the drugs on the painfulness of the immediately preceding electric shocks. The second (fear-plus-pain) session had the succession of shocks of increasing intensities as described above.

The animals were introduced to this procedure by the following stages: On the third day of avoidance training, shock was omitted from the 2-foot alley during the first test session of the day, all other shocks remaining as described above. On the next three days of avoidance training, shock was also omitted from the 3, 4, and 5-foot distances respectively during the first session of each day. On the seventh and last day of training the procedure of the preceding day was repeated: shock (180 volts) was given only in the six-foot alley during the first session. Throughout this period of training, shock was given at all but

the 1-foot distance in the second session of each day, thus preventing the extinction of fear.

Tests with and without drugs. The present study begins with the 2×2 design summarized in Table 1, which is followed by a crossover test in order to allow each *S* to be used his own control.

Group I, trained under a saline placebo, and group II, trained under alcohol, each received a total of 60 avoidance trials (five days at 12 trials per day) before the tests were begun. For the purpose of testing, both groups were divided into halves, yielding groups A and D which received the subsequent tests under the same condition which had been used during training, and groups B and C which were tested under the appropriate drug or control condition not used during the training period. Following this first test, the animals which had been tested under the condition of training were switched (crossover) to the appropriate drug or control condition not yet tested.

The training and testing procedures for group II′, trained under chlorpromazine and control Group I′, trained under a saline placebo, followed a schedule identical to that detailed above for the alcohol tests.

Scores. Two measures were analyzed separately for each drug: Maximum distance to deterrence (MDD), and running speed, the reciprocal of the time scores. For each session, the MDD score is defined as the longest of the series of test alleys in which the rat runs all of the way down and touches the food at the goal. It thus represents the highest level of aversive stimulation the animal will tolerate in order to get food.

Results and Discussion

Alcohol. Let us first consider the effects on test performance of being tested under the influence of alcohol. The results were highly consistent for the two testing conditions, fear and fear-plus-pain. Both performance measures, maximum distance to deterrence (MDD) and running speed, agreed in showing that animals *tested* under alcohol performed better than those tested following the saline injection. The data summarized in Tables 2 and 3 (as well as a similar analysis of the speed scores which is not presented) show that this superiority occurred in both halves of the complete design—i.e. the same alcohol effect appeared both for rats which had received their original training without alcohol (the conventional condition) and also for rats which had received their original avoidance training with alcohol (the other half of the design). Since the effect is not limited to either condition of training, it could not have been caused merely by a stimulus change between the conditions of training and those of testing. The fact that the results were in the same direction in both halves of the design also means that our conclusions are not dependent on any assumption of behaviorally equal

Table 2. *Average distance to deterrence (feet) for fear tests without shock*

Alcohol				Chlorpromazine			
Training	Testing			Training	Testing		
	Alcohol	Control	Sum		Chlor-promazine	Control	Sum
Alcohol	5.5	4.3	9.8	Chlorpromazine	4.2	4.0	8.2
Control	6.0	2.8	8.8	Control	4.8	3.7	8.5
Sum	11.5	7.1	18.6	Sum	9.0	7.7	16.7

Table 3. *Average distance to deterrence (feet) for fear-plus-pain tests with shock*

Alcohol				Chlorpromazine			
Training	Testing			Training	Testing		
	Alcohol	Control	Sum		Chlor-promazine	Control	Sum
Alcohol	4.9	3.0	7.9	Chlorpromazine	3.7	1.3	5.0
Control	5.2	2.5	7.7	Control	3.8	1.3	5.1
Sum	10.1	5.5	15.6	Sum	7.5	2.6	10.1

units of measurement in different parts of the scales used to measure either MDD or speed[1].

To consider the details of these results, the MDD scores in Tables 2 and 3 show that the animals tested under alcohol ran farther and tolerated stronger threats of shock in the fear test, as well as stronger actual shocks in the fear-plus-pain test, than did those tested under saline. Both differences are reliable beyond the 0.001 level.

As Fig. 1 shows, the animals tested under alcohol also ran faster at each of the six distances than did those tested under saline. The difference at each of the six distances in each of the two tests (fear and fear-plus-pain) are all reliable beyond the 0.01 level.

The foregoing clear-cut results, with conditions of previous training (and hence the possible effects of stimulus change) controlled, confirm those previously reported (Masserman and Yum 1946, Conger 1951, Miller and Barry 1960). These is only one minor difference. Miller and Barry (1960) found that at the safe, 1-foot distance, alcohol depressed performance below the placebo level, so that the two speed curves

[1] The reader who is not familiar with the mathematical assumptions underlying an analysis of variance can convince himself of the importance of units of measurement by adding up the marginals and diagonals of a 2×2 table of time scores arbitrarily set up so that there is no interaction (or so that a small difference between the top cells in a pair of columns is offset by a much larger difference between much larger numbers in the bottom pair of cells), and then repeating the same analysis after the same time scores have been changed to speed scores by taking their reciprocals.

actually crossed. CONGER (1951) similarly secured a depressive effect of alcohol in the absence of fear, while in the present study no such depression occurred at the safe, 1-foot distance. Perhaps our rats failed to discriminate clearly between the 1 and the 2-foot alleys and hence had some generalized fear for the alcohol to relieve even at the safe distance.

Now let us turn to a comparison of the animals trained under the influence of alcohol and those trained following a placebo injection.

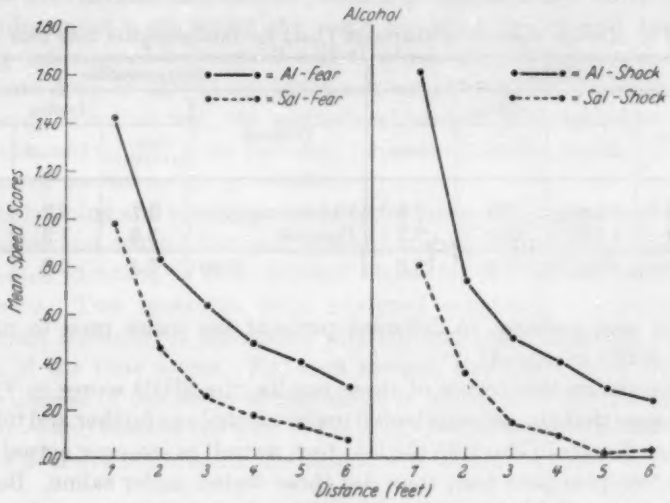

Fig. 1. Running speed in variable-length alley under alcohol and saline control for both "fear-test" and "fear-plus-pain test"

Both the MDD scores and the speed measure (taken separately for each alley-length) agree in showing no differences between these two groups which even approach statistical significance. Apparently, the effects of alcohol during training do not transfer to any marked degree to the subsequent test condition. But if the main effect of alcohol had been an analgesic one, reducing the painfulness of the electric shock, we would expect such transfer. Thus, this lack of generalization fits in with the fact that in the test situation a marked effect of alcohol appeared during the fear session without any shocks (except following the last run), as well as during the fear-plus-pain session, during which the rats received shock at all but the 1-foot distance.

Finally, there were no statistically reliable interactions between the conditions of training and those of testing, but not much emphasis can be placed on this result since its interpretation depends on the assumption that the MDD scores and the speed measures yield equal-interval scales whereas we can only be certain that these scales are related to

the relevant behavioral variables in an ordinal fashion (MILLER 1959, pp. 281–283).

Chlorpromazine. Tables 2 and 3, summarizing the MDD scores for both conditions of testing, show that animals *tested* under chlorpromazine tended to run down longer alleys before stopping, irrespective of whether the original training was with or without the drug. While this difference is highly reliable ($p < 0.001$) for the fear-plus-pain test, it fails to reach statistical significance for the fear test.

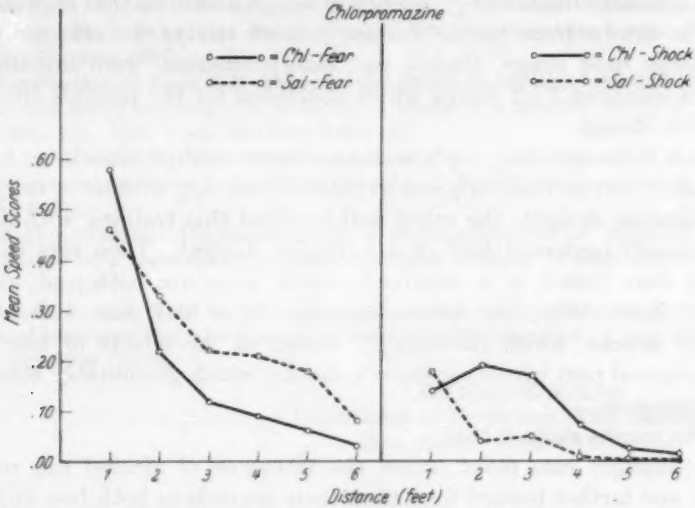

Fig. 2. Running speed in variable-length alley under chlorpromazine and saline control for both "fear-test" and "fear-plus-pain test"

The effects on running speed are shown in Fig. 2. In the fear-plus-pain test chlorpromazine tended to increase running speed, the differences being reliable at the 0.01, 0.01, and 0.02 levels respectively for the 2, 3, and 4 foot distances. These results agree well with the MDD scores. It is easy to explain the lack of a difference at the 1-foot distance as being due to a low level of fear, and the lack of reliable differences at the 5 and 6 foot distances as due to the fact that the shocks are strong enough to stop practically all animals.

On the fear test, however, the differences in speed scores are in the opposite direction and are reliable at the 0.02, 0.05, and 0.01 levels respectively for the 4, 5, and 6 foot distances. They are also in the opposite direction from the difference in the MDD scores on the same test. It appears that in these tests with chlorpromazine some drug-situation interactions may be occurring which we do not understand. BARRY and MILLER (in press) failed to find a differential fear-reducing effect in the telescope conflict alley.

All of the foregoing effects were independent of the conditions of training (with or without chlorpromazine) which did not produce any reliable effects. As in the alcohol tests, none of the interactions between the conditions of training and those of testing showed any reliable effects.

Summary

Virtually all previous studies designed to investigate differential effects of drugs on fear, or aversion, have used an unbalanced design which seriously limits the generality of any conclusions that legitimately may be drawn from them. In the present study, the effects of two commonly used drugs, alcohol and chlorpromazine, were investigated using a balanced 2×2 design which controlled for the possible effects of stimulus change.

In a telescope-alley, approach-avoidance conflict situation, half of the rats received their original avoidance training without a drug (the conventional design); the other half received this training with a drug (the usually-neglected half of a balanced design). Then rats in both halves were tested in a counter-balanced sequence with and without drugs. Each daily test session consisted of a first part without any electric shocks, which presumably measured the effects of fear only, and a second part involving electric shocks, which presumably measured fear-plus-pain.

The results showed that:

(a) Hungry rats *tested* under the influence of alcohol ran reliably faster and farther toward food than their controls in both fear and fear-plus-pain tests. Since similar results appear with rats originally trained with the drug, and also with those originally trained without it, the results are independent of the similarity between conditions of training and testing, and hence could not have been produced by differentially fear-reducing effects of stimulus change.

(b) Administration of alcohol during training had no reliable effect on the performance during testing. This result, as well as the similarity of the drug's effects on both tests—fear only and fear-plus-pain—rules out a possible analgesic effect as the primary basis for an explanation of the differential reduction in aversiveness which it produced.

(c) Rats tested under the influence of chlorpromazine ran consistently farther and *faster* than their controls in the fear-plus-pain tests, but did not run reliably farther and, in fact, ran significantly *slower* than their placebo-controls in the fear-only test. Apparently there was some situation-drug interaction which we do not understand. The foregoing results appeared in both halves of the balanced design. There were no reliable effects of the chlorpromazine during training on the performance during testing.

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The Effect of Drugs on Discrimination and Sensory Generalisation of Auditory Stimuli in Cats

By

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With 3 Figures in the Text

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Introduction

It is a well known fact that indiscriminate arousal does not take place with every sensory stimulus. It is often the meaning of a stimulus, based on past experience, which determines its arousal or attentive value. For a specific tone the intensity of stimulation necessary to produce attentiveness or arousal from sleep is not the same in every animal and in any one animal may fluctuate depending upon the precise environmental conditions or the experimental procedures to which the animal has been subjected (Key and Bradley 1960). By positive conditioning it is possible to increase the significance attached to a particular auditory stimulus and produce a lowering in the intensity of stimulation necessary to evoke arousal. Conversely, during the process of habituation there appears to be a loss of significance associated with a concomitant increase in the arousal threshold for the continually repeated stimulus.

In many ways *d*-lysergic acid diethylamide (LSD 25) appears to simulate the effects of conditioning for a wide range of sensory stimuli. Small doses of this drug produce a decrease in the thresholds for non-conditioned arousal responses, induced by auditory stimuli, both in terms of behaviour and the electrical activity of the cortex, but they do not alter the thresholds for conditioned arousal responses (Key and Bradley 1960). Futhermore, a stimulus to which the animal has previously become habituated evokes a marked response following the systemic administration of LSD 25.

It is the purpose of this study to determine whether or not this apparent increase in responsiveness following the injection of LSD 25 and the differential effect on conditioned and non-conditioned arousal responses is due to the inability of the animal to distinguish between significant and insignificant sensory stimuli, or produced solely by a lack of discrimination between the physical parameters of auditory stimulation employed.

In order to test these possibilities use has been made of conditioned auditory discrimination techniques. In addition, since chlorpromazine is known to antagonise the effects of LSD 25 (BRADLEY and HANCE 1957) and in small doses to produce a progressive increase in all auditory induced arousal thresholds (KEY and BRADLEY 1960), the effect of this drug has also been studied on similar discriminatory problems.

Methods

Sensory Generalisation

Experiments were carried out in a double walled constant environment chamber fitted with a one-way observation window (BRADLEY and ELKES 1953). Eight adult cats were used and the training and testing of the animals were divided into two stages. Firstly, all animals were trained to cross the nine inch high barrier from one side of the constant environment chamber to the other on the presentation of a pure tone of 600 c/sec for five seconds. Failure to accomplish this task within three seconds was punished by an electric shock of 0.5 mA delivered to the feet of the animal from a metal grid. Training was carried out for periods of one to two hours on consecutive days until acquisition of the conditioned avoidance response was established. Each series of conditioning trials was always preceded by a period of acclimatisation to the conditioning environment and the rate at which the conditioning stimulus was presented was not kept constant but varied from one to five per ten minutes.

The animals usually reached the 100% correct response level after 200 to 300 trials and as soon as this level was attained either saline (as a control), chlorpromazine or LSD 25 was injected by the intraperitoneal route. The effects of the drugs were tested 30 minutes later on the extinction rate of the conditioned avoidance response and on the rate of extinction of barrier crossing responses evoked through sensory generalisation by different tones which had not been used during the conditioning trials. The conditioned stimulus was presented in random order with these tones, which were given in sets of eight with a period of one to two minutes between each tone. The intensity of the 600 c/sec conditioning stimulus was kept constant at 40 decibels above an arbitrarily chosen reference level of 1 millivolt into a 3 ohm impedence and the loudness of the neutral stimuli was equated to this level using the audiogram of the cat (DWORKIN, KATZMAN, HUTCHISON and MCCABE 1940). The tones were chosen within the range 200—2000 c/sec so that the effect of notes both higher and lower than the conditioned stimulus could be observed. During these trials no punishment was given for incorrect responses and the tones were presented until extinction of all barrier crossing responses.

The degree of sensory generalisation has been shown to be dependent upon the strength of conditioning (HOVLAND 1937a). Thus, in these experiments the rate of presentation of the stimuli, the parameters of stimulation and the environmental conditions were kept constant and the animals trained irrespective of the number of trials to the 100% correct response level, i.e. the first ten correct responses out of ten consecutive trials, and the conditioning was stopped as soon as this level was reached. Since the animals were used for more than one experiment it was expected that alterations in the rate of extinction of the responses might occur due to familiarisation with the experimental procedure. In order to obtain some assessment of these changes in relation to the effects of the drugs the experiments with each cat were carried out in a control, drug, drug, control sequence. Moreover, since chlorpromazine is known to produce alterations in the rate of extinction of a conditioned response (ADER and CLINK 1957), which may effect subsequent reconditioning rates the first drug to be given in the series was always LSD 25.

The results for each animal were expressed as the graph of generalisation of extinction by plotting the number of trials to extinction of each tone against its frequency. The degree of generalisation may be expressed as the gradient of the graph of generalisation of extinction when the auditory stimuli are scaled along a single psychophysical dimension, in this case pitch. The relationship between pitch and frequency, however, has not been worked out for the cat. Thus the data plotted for each animal was in the form of a composite graph of two components, the gradients of which differed, due to a Weber-Fechner effect, depending upon whether or not the tones were greater or lesser in frequency than that of the conditioned stimulus. For the purposes of analysis each component, i.e. frequencies between 200 to 600 c/sec and 600 to 2000 c/sec, were taken separately. Linear regression formulae, given by the term $y = a + b(x - \bar{x})$, were determined for each animal using suitable transformations ($\sqrt{y} = x$, and $y = 1/x$, respectively) and the differences produced by the drugs in the rate of extinction of the barrier crossing responses (given by a, the displacement of the graph) and the degree of sensory generalisation (given by b, the gradient of the graph) tested using the 'Student' t test for correlated data.

Auditory Discrimination

Method 1. The animals were also trained to discriminate between two tones of equal intensity and duration. For one tone (Tone A) they were required to jump the hurdle and for the other tone (Tone B) they had to remain where they were. There is a variety of techniques for training animals to distinguish between sensory stimuli of one modality but the one most widely used is that termed the 'Method of Contrasts'

(Hilgard and Marquis 1940). For this technique use is made of the phenomenon of sensory generalisation, since the responses evoked in this manner are more easily extinguished than the conditioned response. Thus discrimination can be produced by non-reinforcement of the generalised response while reinforcement of the conditioned response is continued. Eight cats were trained to discriminate between the two tones using this method, here termed Method 1, and the effects of chlorpromazine and LSD 25 were studied on the response.

Method 2. Subsequently, the animals were retrained using the same avoidance response situation as described above to discriminate between two other tones. This time the experimental procedure was modified and both auditory stimuli were reinforced, so that any incorrect response was punished by a mild electric shock delivered to the paws of the animal. When training was complete and a 90 to 95% correct response level had been attained the same procedure was adopted as in Method 1, namely, normal saline was injected as a control and 30 minutes later the animal tested over 20 non-reinforced trials. The stimuli, which were presented at the rate of one every two minutes, were given in random order so that during the control period there were 10 presentations of each tone. LSD 25 or chlorpromazine was then injected by the intraperitoneal route and the animal retested 30 minutes later, using the same procedure. One week was allowed to elapse for complete recovery from the effects of the drug and the animal again tested over a further 20 trials but this time without any injection.

Since each animal was taken as its own control, the effects of the drugs were assessed by employing a one-tailed sign test on the sign of the differences between the correct response scores before and after the administration of the drugs.

Results

The effect of drugs on generalised avoidance responses

The extinction of sensory generalised barrier crossing responses evoked by tones within the frequency range of 200 to 2000 c/sec was studied after the establishment of a conditioned avoidance response to a tone of 600 c/sec. The results are summarised graphically in Fig. 1, where data from all the animals has been pooled and the mean number of trials to extinction of each tone has been plotted against frequency in c/sec. The curves show that in the cat the number of non-reinforced trials needed to produce extinction of the generalised responses decreased with increasing difference in frequency from that of the conditioned stimulus. The greatest decrease in the gradient of generalisation of extinction occurred with only slight changes in frequency from 600 c/sec but even with gross differences there was still a significant amount of generalisation.

Comparison of the control graphs (Fig. 1) showed that although the differences which existed in the rates of extinction, before and after the drug treatments, were not significant ($P = 0.3$—0.2), there was a tendency in the second control for the rate of extinction to increase slightly. However, the expected modification produced by successive extinction of the same response appeared to have been eliminated by the interposition of the drug treatments. The degree of generalisation, given by the slope of the graphs, was also not significantly altered by familiarisation

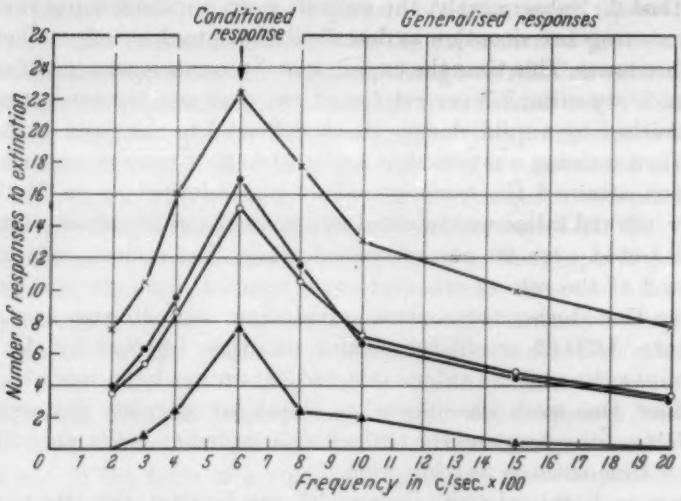

Fig. 1. The effect of drugs on the extinction of a conditioned avoidance response and qualitatively similar responses evoked through sensory generalisation. • First control. × 15 μg/kg of d-lysergic acid diethylamide i. p. ▲ 5 mg/kg of chlorpromazine i. p. ○ Second control

with the experimental procedure over the limited number of experiments carried out with each animal.

The effect of LSD 25 on the rate of extinction was marked (Fig. 1) and, following the intraperitoneal injection of 15 μg/kg., not only was there an increase in the number of trials required to extinguish the conditioned response but also a significant increase ($P = < 0{\cdot}001$) in the number of trials necessary to produce extinction of the sensory generalised responses in all animals. This decrease in the extinction rate, associated with the administration of LSD 25, was in complete contrast to the effect produced by chlorpromazine. After a dose of 5 mg/kg i.p. of this drug, all responses were extinguished more rapidly ($P = < 0.001$). For example, where previously 11 to 18 sets of tones were required for total extinction of all barrier crossing responses, following chlorpromazine only 6 to 9 were needed (Fig. 1). This effect was not accompanied by any signs of locomotor deficit or sedation, since the animal would still cross the barrier readily when the shock was presented alone.

Analysis of the regression coefficients of the graphs for each animal indicated that the gradients of generalisation were not significantly altered by either 15 μg/kg of LSD 25 or 5 mg/kg. chlorpromazine. Thus the degree to which the conditioned response was generalised to other auditory stimuli remained unchanged but, since the rate of extinction of the conditioned response was modified both by LSD 25 und chlorpromazine, the amount of generalisation, that is the number of tones capable of eliciting generalised responses, altered accordingly (Fig. 1). For example, after chlorpromazine the animals failed to respond to auditory stimuli of 200 and 2000 c/sec. although in the control these tones elicited barrier crossing responses for at least the first two to five trials.

The effect of drugs on auditory discrimination

A conditioned avoidance response involving discrimination between two auditory stimuli of equal intensity and duration was established using two different methods of training. Although the avoidance response was the same in each procedure the patterns of behaviour noted during the conditioning trials were not the same. This variation, which may have a bearing on the interpretation of the effects produced by LSD 25 may be illustrated graphically by considering the rates of learning for one of the animals under the two types of procedure (Fig. 2).

Method 1. The cat was conditioned to jump the barrier on the presentation of Tone A (600 c/sec). No other auditory stimulus was given at this stage until a 70 to 80% correct response level had been attained. Another tone of 400 c/sec, as Tone B, was then substituted in 50% of the trials. For some time the animal continued to cross the barrier to both tones, but by reinforcing the 600 c/sec note and not the 400 c/sec tone, the animal gradually learned to distinguish between the two and eventually would only carry out an avoidance response when the 600 c/sec tone was presented. This is shown by the steady rise to the 100% correct response level in the latter part of the graph in Fig. 2a.

At this stage in the conditioning a control injection of normal saline had no measurable action on the conditioned response but following 5 mg/kg of chlorpromazine the discrimination between tones A and B was apparently lost. All animals, without exception, rapidly failed to make the right response when tone A was presented and finally, after 5 to 10 trials, they stopped crossing the barrier altogether. Thus a lower mean correct response level of approximately 65% was recorded (Fig. 3a). On the other hand, after 15 μg/kg of LSD 25 the animals not only continued to respond to Tone A but also started to cross the barrier indiscriminately to Tone B. The incidence of barrier crossing responses therefore increased following the administration of this drug and resulted

in a correct response score of between 60 to 65%, which was again lower than the control levels (Fig. 3b). Upon recovery from the effects of the LSD 25 the conditioned discrimination response returned to control, or near control levels, whereas following the chlorpromazine experiments the correct response score was usually only just above the 50% chance level (Fig. 3a).

Method 2. Reinforced, random presentation of one or the other of the tones from the very beginning of the conditioning trials resulted in

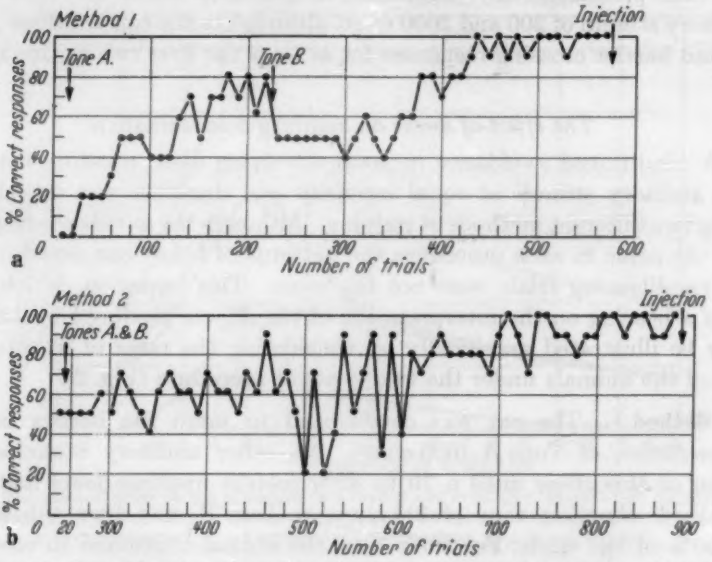

Fig. 2a and b. Rate of learning of a conditioned auditory discrimination response. *Method 1* by reinforcing only one of the tones (*Tone A*), *Method 2* by reinforcing both tones

the development of a different behavioural pattern from that already described. Initially the animal did not cross the barrier until the punishing shock was administered, but as an association between the shock and the auditory stimulus was established, the animal began to carry out avoidance to both tones indiscriminately. As a result it received punishment in 50% of the trials when it arrived on the other side of the barrier. At this stage the animal became extremely excited, more so than during the training in Method 1, and presentation of either of the tones produced hissing, spitting and vocalisation, followed usually by the animal attacking the barrier. In three cases the behaviour of the animals became completely abnormal and conditioning had to be abandoned. These animals appeared perfectly normal in their home cages but immediately on returning to the conditioning environment the abnormal behaviour again developed.

Most animals however, appeared to notice some difference between the two stimuli after further trials, resulting in a period when they either responded correctly in 70 to 90% of the trials or failed to get more than 20 to 30% right. This stage is shown on the graph (Fig. 2b) by the large

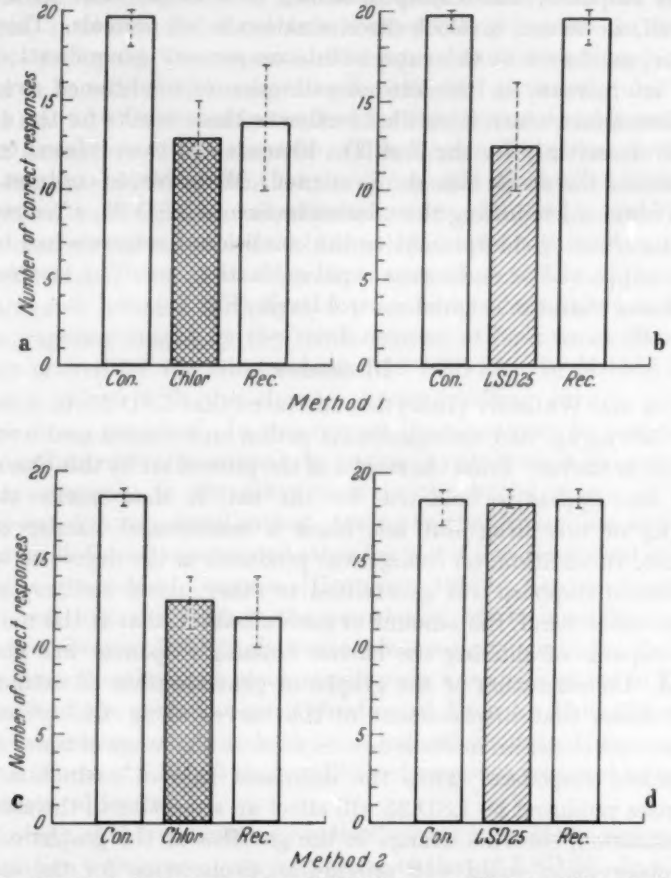

Fig. 3a—d. The effect of chlorpromazine and LSD 25 on a conditioned auditory discrimination response taught in *Method 1* by reinforcement of only one tone and in *Method 2* by reinforcement of both tones. *Con.* Control. *Rec.* Recovery. *Chlor* 5 mg/kg Chlorpromazine i.p. *LSD 25* 15 μg/kg d-lysergic acid diethylamide i.p. The number given is the mean correct response score in twenty consecutive trials for all the animals. Dotted lines indicate experimental variation

oscillations, a feature characteristic of the five animals which successfully underwent this type of training. Following this stage, acquisition of the conditioned avoidance response to the right stimulus was quickly attained although it was never possible to obtain more than a 90 to 95% correct response level and, compared with Method 1, the rate of learning was much slower.

The effects of LSD 25 and chlorpromazine on the conditioned discrimination response taught by Method 2 are shown in Fig. 3c and d. In the control and drug trials no reinforcement was given and after an injection of 15 μg/kg of LSD 25 there was no marked change in the level of response, but chlorpromazine, in a single dose of 5 mg/kg appeared, as before, to block discrimination in all animals. This drug, however, as shown in the experiments on sensory generalisation, produced an increase in the rate of extinction of conditioned avoidance responses, a fact which is verified further in these results for the animals responded correctly for the first 5 to 10 trials and then refused to move at all unless the shock was also presented. Moreover, in contrast to the results obtained following the administration of LSD 25, after recovery from the effect of chlorpromazine the conditioned response had in most cases disappeared or underwent rapid extinction, resulting in a response score lower than the original control levels (Fig. 3c).

Discussion

Cook and Weidley (1957) demonstrated that LSD 25, in doses from 100 to 500 μg/kg, had no measurable action on a conditioned avoidance response in the rat. From the results of the present study this observation would also appear to hold true for the cat, in that smaller doses of 15 μg/kg of this drug did not block a conditioned barrier crossing response. In addition, no change was produced in the degree to which a conditioned response was generalised to other, novel auditory stimuli. On the other hand, the amount of generalisation, that is the number of tones capable of eliciting the barrier crossing responses was markedly altered. Consideration of the graphs of generalisation of extinction in Fig. 1 shows that displacement of the curves along the 'y' ordinate will produce either an increase or a decrease in the range of tones evoking generalised responses. Thus the decreased rate of extinction of the responses produced by LSD 25 will effect an alteration in the amount of generalisation, since no change in the gradient of the graphs occurred. This observation could well provide an explanation for the increased responsiveness of animals to sensory stimuli after LSD 25 (Bradley and Elkes 1957) and the ability to awaken sleeping animals with stimuli which before the administration of the drug had failed to evoke an arousal response (Key and Bradley 1960).

Changes in responsiveness could, of course, be produced by alterations in auditory acuity, but the fact that LSD 25 does not produce any change in the threshold intensities of auditory stimulation necessary to evoke conditioned behavioural and electrocortical arousal responses (Key and Bradley 1960) would argue against this hypothesis. A possible explanation for the decreased rate of extinction may be that LSD 25 increases

the meaningfulness or significance level of the sensory stimuli. For example, the rate of extinction of a conditioned response is dependent, to a certain extent, upon the degree of conditioning. In conditioning trials as a tone/shock situation is repeated the psychological significance or meaning, in this case the fear of anxiety attached to the auditory stimulus, increases and there is a corresponding increase in the time taken to extinguish the response. The rate of extinction therefore, may be said to be related to the significance attached to the stimulus by the animal and any alteration in the level of significance is mirrored in the rate of extinction of the response. This hypothesis, although difficult to substantiate, could account for the differential effect of LSD 25 on the conditioned auditory discrimination responses. Obviously the blocking of the conditioned response established by reinforcing only one of the tones (Method 1) cannot be dependent upon the inability of the animal to distinguish between the physical parameters of stimulation, since a similar response taught by the reinforcement of both tones (Method 2) was not altered by the administration of LSD 25. In Method 2 both tones are paired with the electric shock, resulting in any incorrect response being punished. In this respect the tones may be said to have equal significance for the animal. In Method 1 only Tone A was reinforced and any response to the other tone in the initial stages of conditioning was due to generalisation. Owing to the differential extinction rates of a reinforced conditioned stimulus and a non-reinforced stimulus evoking a generalised response (HOVLAND 1937b) barrier crossing to Tone B was lost and with it the association of the punishing shock. Under the effect of LSD 25, however, since no change in the degree of sensory generalisation took place, significance was again added to Tone B as a result of the spread of generalisation of Tone A. Thus the animal started to cross the barrier to both tones indiscriminately. Upon recovery from the effects of LSD 25 the conditioned response returned to control or near control levels.

The effect of chlorpromazine on discrimination problems and sensory generalisation was in many ways opposite to that of LSD 25. In a single dose of 5 mg/kg, a dose which produced little sedation and no motor deficit, two effects were observed. Firstly, the rate at which the conditioned, as well as the generalised responses, were extinguished was significantly increased. Secondly, although the gradient of generalisation remained unaltered, the range of tones eliciting barrier crossing responses was markedly reduced. The ability of chlorpromazine to block conditioned avoidance behaviour has been demonstrated previously by numerous workers. ADER and CLINK (1957) established that this drug will affect the acquisition, or produce rapid extinction of a conditioned avoidance response in rats. MILLER, MURPHY and MIRSKY (1957a) also

found a more rapid extinction of avoidance responses following chlorpromazine, which by comparison with a phenobarbital control group was found to be independent of sedational effects or motor impairment. Similarly, chlorpromazine will produce rapid habituation of arousal response, both in terms of behaviour and the electrical activity of the cortex (KEY 1961).

The apparent rapid relearning which takes place under chlorpromazine offers an explanation of the blocking action of this drug on the conditioned auditory discrimination responses. The fact that changes in frequency discrimination are not involved is evidenced by the way the animals were able to distinguish between the two tones and respond at the 100% correct response level for the first 5 to 10 trials. Moreover, in agreement with the results of MILLER, MURPHY and MIRSKY (1957 b) in rats, spontaneous recovery of the conditioned response following the chlorpromazine experiments did not occur. Thus the eventual blocking of this response at a dose level of 5 mg/kg must be a consequence of the increased rate of extinction, an effect similar to that reported for conditioned and non-conditioned auditory-induced arousal responses (KEY and BRADLEY 1960).

In conclusion, it would appear that LSD 25 is able to alter the level of significance or meaningfulness of stimuli, thereby producing an increased amount of generalisation without modifying the discriminatory ability of the animal in terms of the physical parameters of stimulation. Chlorpromazine produces the opposite effect. The amount of generalisation is less and stimuli lose the significance attached to them by the animal, resulting in the indifference and lack of responsiveness to sensory stimuli characteristic of the central action of this drug.

These results may also have some bearing on the effects induced by chlorpromazine and LSD 25 in man. Generalisation and discrimination play important roles in perception. Generalisation is important in adaptive ability, since environmental situations never recur without some modification. The significance or meaning attached to one stimulus may be generalised to a wide range of sensory stimuli or in some cases restricted to quite a narrow range. If, by the administration of certain drugs such as LSD 25, the level of significance of the sensory information is altered, thus inducing changes in the balance of generalisation, then distortions of perception may occur, for the animal now responds, pays attention or arouses to stimuli which normally would not produce such an effect.

Summary

The effects of lysergic acid diethylamide and chlorpromazine have been studied on generalisation and discrimination of auditory stimuli. LSD 25 (15 μg/kg) produced a significant decrease in the rate of extinc-

tion of a conditioned avoidance response and, although not modifying the degree to which the conditioned response was generalised to other, novel auditory stimuli, elicited a marked effect on the number of tones capable of evoking barrier crossing responses. LSD 25 also appeared to block a conditioned auditory discrimination response which had been established by reinforcing only one of the tones, but failed to exert any significant effect on a similar response taught by reinforcing both tones.

Chlorpromazine (5 mg/kg) produced rapid extinction of conditioned and generalised responses without altering the gradient of generalisation. The number of tones capable of evoking avoidance responses, however, was significantly reduced. A possible explanation of these results has been discussed.

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Potentiation of the Behavioral Effects of Amphetamine by Imipramine* **

By

PETER L. CARLTON

With 5 Figures in the Text

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We have previously reported that two parasympatholytics (atropine and scopolamine) significantly augmented the tendency of amphetamine to increase the response rates of rats working in an operant, shock-avoidance situation (CARLTON and DIDAMO; CARLTON). This report describes a similar action of a third drug, imipramine[1], which has, relative to atropine and scopolamine, low parasympatholytic potency (SIGG).

The interaction of imipramine and amphetamine was studied in an operant, shock-avoidance situation of the type used in our previous experiments on the effects of atropine and scopolamine in combination with amphetamine. In a subsidiary experiment, the effects of imipramine and amphetamine, singly and in combination, were cursorily evaluated in a food-reinforcement situation.

Materials and methods

Subjects. Ten adult, male, Sprague-Dawley rats were used. Eight of the animals were used in the avoidance experiments and had continuous access to food and water except during experimental sessions; two were used in the food reinforcement experiments and were maintained at 75% of their *ad lib* weights. Each rat had had extensive training on its respective schedule prior to the beginning of the evaluation of the drugs.

Apparatus. The avoidance animals worked in a standard, sound-insulated response chamber which contained two lever-operated switches and a grid floor through which brief electric shocks could be delivered. The dimensions of the chamber were $7\,^3/_8 \times 12 \times 9\,^1/_2$ inches. The "food" animals worked in a similar chamber, which contained, in place of the levers, a 1-inch square plastic plate (the response "key") mounted in

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** A portion of these data was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, 1960.

[1] Tofrānil; generously supplied by Geigy Pharmaceuticals, Ardsley, New York.

one wall of the chamber 1 inch above the floor. A small microswitch was mounted behind the key; each displacement of the key was recorded as the closure of this switch. Below the key, and at floor level, there was a small opening through which the cup of a motor-driven dipper could be presented. With each operation of the motor, the cup was raised from the tank of milk in which it rested, was presented to the animal through the opening for about 3 seconds, and was returned to the tank. The general features of this type of apparatus have been described elsewhere (FERSTER and SKINNER). A system of relays, timers, and counters automatically programmed the experiments and recorded responses to the levers or to the key, deliveries of shock, and dipper operations. In addition, Gerbrands cumulative recorders provided continuous records of the animals' responding.

Avoidance procedure. The animals were trained to depress one of the response levers with their forepaws in order to avoid shock. The avoidance schedule used has been detailed previously (SIDMAN). The essential component of the schedule was a recycling timer set to deliver an inescapable shock of about 1.5 ma intensity and 0.25 second duration every 20 seconds. The circuit was arranged so that each depression of one of the levers in the response chamber reset the timer. After each reset, the timer started again and, in the absence of a subsequent response, delivered the shock at the end of 20 seconds. Thus, any 20-second period in which a lever depression did not occur terminated with delivery of shock, whereas each response reset the timer and thereby postponed the shock. An animal that depressed the lever at a rate no lower than 1 per 20 seconds never received a shock; one that never pressed the lever was shocked every 20 seconds. Each of the animals developed a stable response rate between these two extremes. Depressions of the second lever in no way influenced the delivery of shock.

Reinforcement Procedure. The animals were reinforced with milk only when they displaced the response-key no less than 15 seconds after the preceding response. (They had previously been trained to displace the key by pushing against it with their noses.) Thus, the animals were differentially reinforced for responding at a low rate; an animal that never "paced" its responses at a rate of one/15 seconds or less was never reinforced. Both of the animals adopted a stable response rate that approximated that imposed by the schedule of reinforcement.

An additional component of the schedule was a non-reinforcement period ("time out") in which no response could operate the dipper. When the differential reinforcement schedule was in effect the response chamber was illuminated; when the non-reinforcement component was in effect the light was off. Fifteen-minute periods of reinforcement and time-out were alternated throughout the $4\frac{3}{4}$ hours of the session. Since

these sessions began and ended with the reinforcement component in effect, there were 10 reinforcement and 9 time-out periods of 15 minutes each.

General procedure. Each of the avoidance animals underwent two experimental sessions per week. The experimental session began immediately after the animal had been placed in the response chamber. After 1 hour the animal was removed, given either saline or drug injections, and replaced in the apparatus for 5 hours. Each animal received one control and one drug session per week. A drug was not given oftener than once a week.

The drugs, imipramine hydrochloride and *d*-amphetamine sulfate, were dissolved in normal saline and so diluted that the required dose per kg was contained in 1 ml. In each session the rats were given two intraperitoneal injections no more than 1 minute apart. The following pairs of doses were given to the avoidance animals: saline + saline (control), imipramine + amphetamine, saline + amphetamine, or imipramine + saline. The actions of two imipramine doses (5.0 and 10.0 mg/kg) and of two amphetamine doses (0.5 and 1.0 mg/kg) were studied. The effects of the two imipramine doses and of 1.0 mg/kg amphetamine were studied in all avoidance animals; the lower amphetamine dose was used in only three. The various drug combinations were given in an irregular order. All doses have been given in terms of the weights of the salts. From one to four determinations of the effects of each of the drug combinations were made for each animal; each rat received five to fifteen saline + saline (control) sessions. The data from multiple determinations have been given as averages.

The animals working in the differential reinforcement situation were allowed 30 minutes (one reinforcement and one non-reinforcement period) exposure to the schedule, removed and given two intraperitoneal injections, and returned to the response chamber for the remaining $4^1/_4$ hours of the session. Each animal received one of the following pairs of doses: saline + saline, saline + 6.25 mg/kg imipramine, saline + 12.5 mg/kg imipramine, 1.0 mg/kg amphetamine + saline, 1.0 amphetamine + 6.25 imipramine, or 1.0 amphetamine + 12.5 imipramine. One to four determinations of the effects of each dose combination were made for each animal.

Results

Avoidance. A representative set of cumulative response records for Rat S-18 has been given in Fig. 1. The recording pen was reset to the base line after each 20-minute period and after the accumulation of 500 responses. Shock deliveries have been indicated in the recording channel below each record. The records have been vertically arranged in 1-hour segments for each of the dose combinations (saline + saline,

10.0 mg/kg imipramine + saline, saline + 1.0 mg/kg amphetamine, and 10.0 imipramine + 1.0 amphetamine). The figure indicates that whereas imipramine + saline failed to increase response rate, this same dose of imipramine produced a marked increase when given in combination with

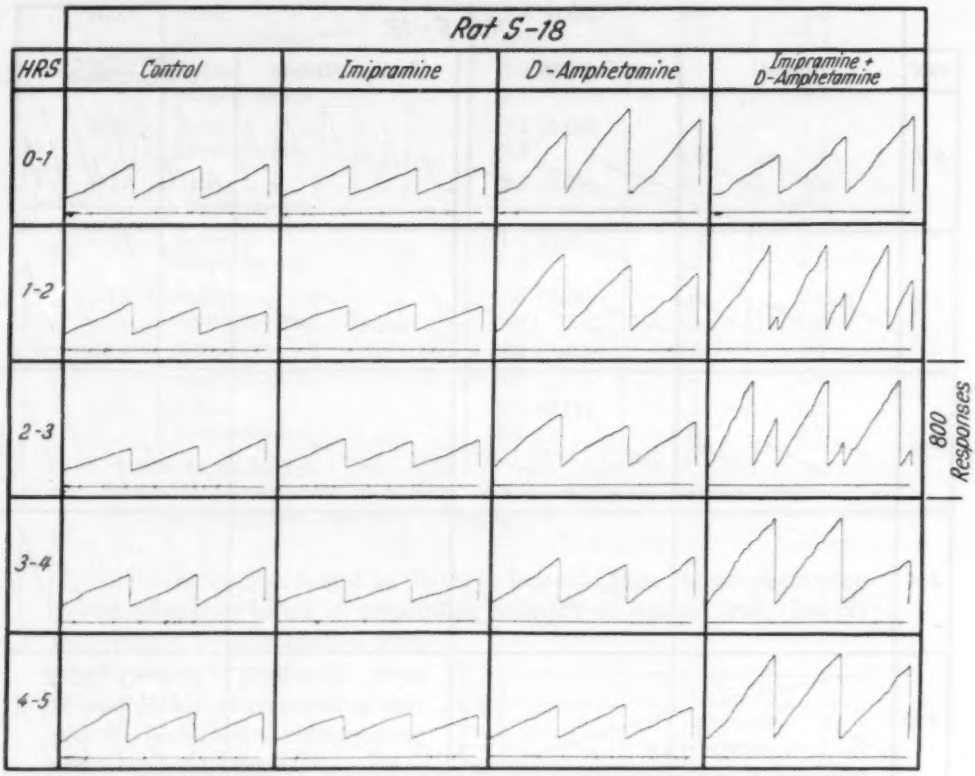

Fig. 1. Cumulative response versus time records from individual sessions for Rat S-18 following control (saline + saline), imipramine + saline, saline + amphetamine, and imipramine + amphetamine injections. See text for doses. Because avoidance responses are plotted cumulatively, the slopes of the response records are proportional to the rate of responding; shock deliveries have been tallied in the recording channel below each record

1.0 mg/kg amphetamine. Further, the increase was greater than that recorded following 1.0 mg/kg amphetamine + saline. In particular, the combination of the two drugs produced a greater "peak" response value (maximum number of responses in any 20-minute period) as well as a markedly prolonged period of super-normal responding. The maximum response rate was reached somewhat later following imipramine + amphetamine than after saline + amphetamine.

A similar effect is shown in Fig. 2. In this instance the increase in the response maximum and the prolongation of the period of increased

responding were even more marked than for Rat S-18. Again, the maximum response rate was attained later when imipramine (10.0 mg/kg) and amphetamine (0.5 mg/kg) were given than when saline and amphetamine (0.5 mg/kg) were given.

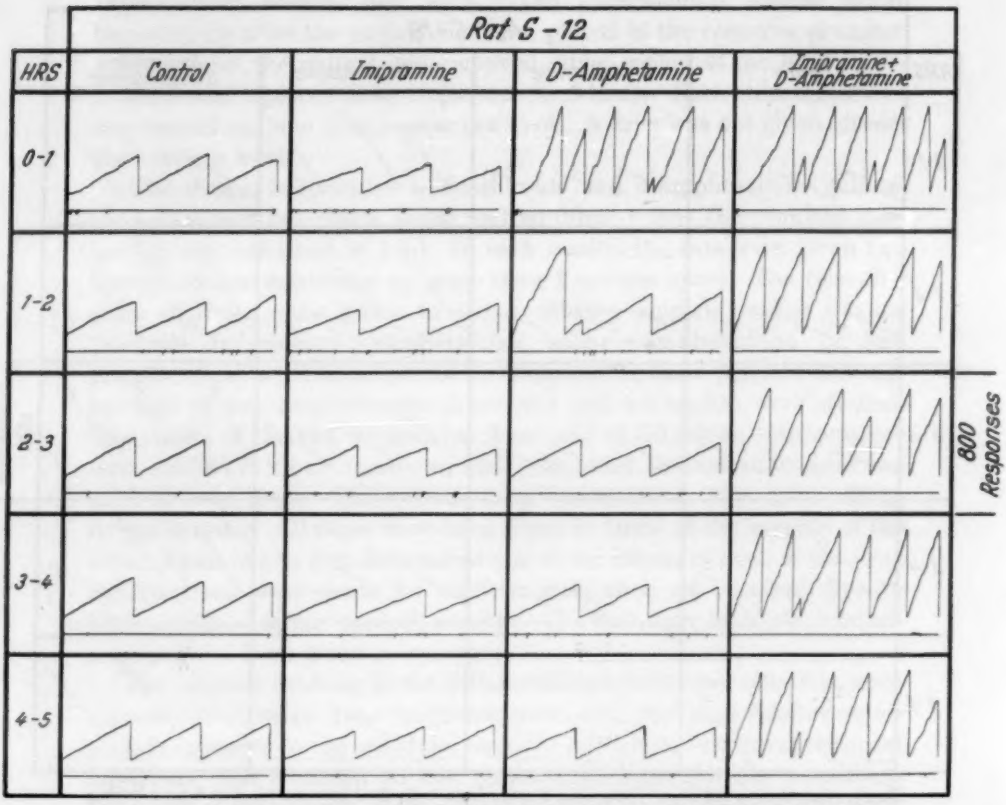

Fig. 2. Cumulative response versus time records for Rat S-12 following control (saline + saline), imipramine + saline, saline + amphetamine, and imipramine + amphetamine injections. See text for doses. The records have been arranged as in Fig. 1

The averages of the total number of responses emitted in the five hours of the post-dose period for all animals at each dose combination (except those involving 0.5 mg/kg amphetamine) have been plotted in Fig. 3. The imipramine doses have been given on the abscissa; "zero" imipramine is saline. The value plotted at 0 on the imipramine + saline curve is the average from the saline + saline sessions, whereas the value at 0 on the imipramine + amphetamine curve is from the saline + amphetamine sessions. The data summarized in Fig. 3 have been entered in Table 1.

Table 1. *Average total post-dose responses* (in thousands)

Rat	First injection	Second injection		
		Saline	Imipramine (5.0)*	Imipramine (10.0)
S-00	Saline	2.8 (0.09)**	3.6	2.6
	Amphetamine***	4.0	5.5	8.7
S-01	Saline	2.3 (0.11)	1.8	1.7
	Amphetamine	3.1	4.2	5.3
S-03	Saline	2.1 (0.06)	1.7	1.9
	Amphetamine	2.9	2.9	6.2
S-12	Saline	2.9 (0.09)	2.3	2.5
	Amphetamine	7.4	11.6	10.2
S-15	Saline	1.8 (0.09)	2.0	1.7
	Amphetamine	2.2	4.2	4.5
S-17	Saline	2.5 (0.04)	2.4	2.6
	Amphetamine	2.9	5.4	5.9
S-18	Saline	3.0 (0.07)	2.5	2.2
	Amphetamine	4.3	5.2	6.8
S-23	Saline	2.8 (0.11)	2.5	2.2
	Amphetamine	5.1	9.0	9.7

* Dose in mg/kg, i.p.
** Standard error of the mean for all saline + saline (control) sessions.
*** All amphetamine doses were 1.0 mg/kg, i.p.

The data in Fig. 3 and in Table 1 indicate that (1) amphetamine produced higher levels of responding, relative to control, and that (2) imipramine in combination with amphetamine produced even greater levels of responding despite the fact that (3) imipramine alone tended to *decrease* response rate (note, however, S-00 after 5.0 mg/kg imipramine). These data were statistically analyzed in the following way: for each *pair* of dose combinations (saline + saline —A— and saline + amphetamine —B—, for example) the A value was subtracted from the B value for each rat and the mean of these differences for all rats was computed. The mean was then evaluated by a *t*-test under the hypothesis that the mean of the differences was zero (LEWIS).

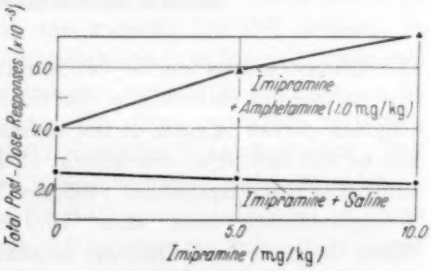

Fig. 3. Dose response curves based on the averaged total numbers of post-dose responses (in thousands) for all animals

These analyses indicated that, when compared with the saline + saline condition, 5.0 mg/kg imipramine did not alter responding, whereas

10.0 mg/kg produced a slight but significant ($p<.02$) reduction. Amphetamine produced, relative to saline + saline, an increase in responding ($p<.02$). This increase was significantly potentiated by the concurrent administration of imipramine; when compared with the number of responses emitted following saline + amphetamine, the numbers following both 5.0 mg/kg and 10.0 mg/kg imipramine + amphetamine were found to be significantly greater ($p<.01$ and $p<.001$, respectively).

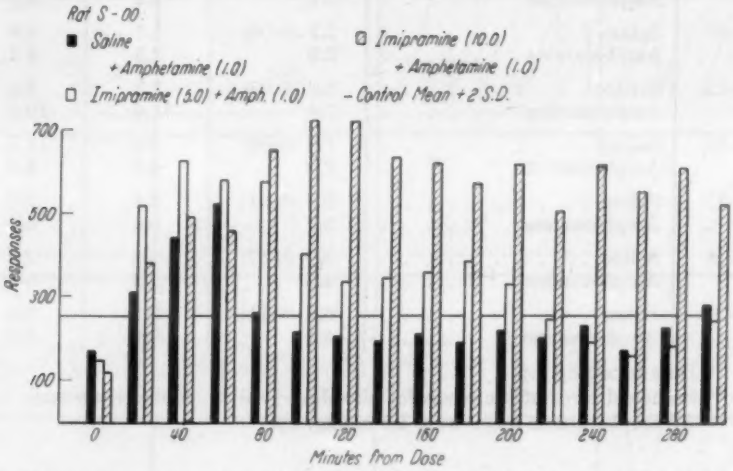

Fig. 4. Numbers of responses in each of the 20-minute periods following the dose-combinations given in the legend at the top of the figure. The values plotted at zero were taken from the last 20 minutes of the predose periods. The values have been averaged from the data obtained in sessions at each dose-combination for one animal

The difference between the two imipramine + amphetamine dose combinations was not statistically significant ($p<.10$). Inspection of the dose response curves of each of the animals suggested that this was largely due to the fact that one animal (S-12) emitted a considerably greater number of responses (about 1400; see Table 1) after 5.0 mg/kg imipramine + amphetamine than after 10.0 mg/kg imipramine + amphetamine. When the data from this one animal were excluded from the analysis, the difference between the effects of 5.0 and 10.0 mg/kg imipramine + amphetamine was found to be significant ($p<.02$). Several of the characteristics of the dose-response curves of other individual animals will be discussed below.

A somewhat more sensitive way of treating the post-dose responding is illustrated in Fig. 4. This figure shows the number of responses emitted in each of the successive 20-minute intervals of the post-dose period for Rat S-00 following the saline + amphetamine and the two imipramine + amphetamine dose combinations. The three sets of data

differ with respect to (1) the maximum number of responses (in any 20-minute interval) as well as to (2) the duration of the period of super-normal responding.

The horizontal line at about 250 responses in Fig. 4 equals th e contro mean + 2 S.D. This animal underwent five control (saline + saline) sessions. The mean and S.D. computed from each of the fifteen 20-minute periods for all five sessions (i.e., 75 numbers entered into the computations) were 189 and 33, respectively. Thus, any response value for a 20-minute period greater than 255 (189 + 66) could reasonably be attributed to the drugs and not to random variations in responding. The duration of the period of super-normal responding was arbitrarily defined as the number of 20-minute intervals after dosing in which the level of responding was greater than the control mean + 2 S.D. Thus, the duration value assigned for amphetamine was 5; for 5.0 mg/kg imipramine + amphetamine, 10; and for 10.0 mg/kg imipramine + amphetamine, 15 (i.e., 5 hours). It should be emphasized that duration values of 15 probably under-estimate the true duration since they correspond to super-normal values for 5 hours, the time at which all experiments were terminated.

The maximum numbers of responses and the duration values following amphetamine and imipramine + amphetamine have been entered in Table 2. In most cases, both the response maxima and the duration values showed a dose-dependent relation to imipramine. The response maxima tended to reflect this dependency less reliably than did the duration values. The inversion in the dose-response curve for S-12, mentioned previously, is shown in the maxima for this animal. In comparing the 0.5 and 1.0 mg/kg amphetamine data for S-18, it should be noted that the duration values indicate very little potentiation by imipramine + 0.5 mg/kg amphetamine; the total post-dose responses were, however, slightly greater at 10.0 imipramine + amphetamine. This was apparently due to the atypically high duration value at saline + 0.5 mg/kg amphetamine. The data for S-03 should be noted; in terms of both maxima and duration values, 5.0 imipramine failed to augment the effects of 1.0 amphetamine.

The data presented in Figs. 1, 2, and 4 suggested that the maximum response values following imipramine + amphetamine were not only greater than those following amphetamine + saline but also occurred later in the post-dose period. This was generally true for all animals; response maxima following 1.0 mg/kg amphetamine were recorded, on the average, 56 minutes after dosing, 90 minutes after 5.0 mg/kg imipramine + 1.0 amphetamine, and 130 minutes after 10.0 mg/kg imipramine + 1.0 amphetamine.

Table 2. *Response maxima and duration values following amphetamine and imipramine plus amphetamine*

Rat	First injection	d-Amphetamine (second injection)			
		maximum Rs/20 min		No. intervals > contr.	
		0.5 *	1.0 *	0.5	1.0
S-00	Saline	—	520.0	—	5
	Imipramine (5.0)**	—	626.0	—	10
	Imipramine (10.0)	—	722.0	—	15
S-01	Saline	—	324.0	—	8
	Imipramine (5.0)	—	374.7	—	13
	Imipramine (10.0)	—	453.0	—	14
S-03	Saline	222.0	274.0	4	8
	Imipramine (5.0)	—	272.0	—	7
	Imipramine (10.0)	305.0	533.0	15	14
S-12	Saline	1082.0	1029.0	6	10
	Imipramine (5.0)	—	1002.0	—	15
	Imipramine (10.0)	1102.5	904.0	15	15
S-15	Saline	—	186.0	—	5
	Imipramine (5.0)	—	473.0	—	13
	Imipramine (10.0)	—	406.0	—	14
S-17	Saline	—	384.0	—	2
	Imipramine (5.0)	—	470.0	—	14
	Imipramine (10.0)	—	505.0	—	15
S-18	Saline	346.0	448.0	10	7
	Imipramine (5.0)	385.0	430.0	11	15
	Imipramine (10.0)	360.0	639.5	11	14
S-23	Saline	—	721.0	—	8
	Imipramine (5.0)	—	994.0	—	15
	Imipramine (10.0)	—	907.0	—	14

* Amphetamine doses in mg/kg.
** Dose in mg/kg.

Food reinforcement. The effects of the various drug combinations were evaluated in terms of three post-dose measures: (1) the total number of responses emitted during the reinforcement periods, (2) the number emitted during time-out (non-reinforcement) periods, and (3) the median inter-response times of the reinforcement periods. These last values were calculated from the frequency distributions of inter-response times (the times between successive responses) tabulated by 3-second intervals on a special system of counters and programming equipment. After a sequence of five responses in which the successive inter-response times were 10, 3, 16, and 18 seconds, for example, there would be one count in the 9—12 second category, one in the 0—3 second category, and two in the 15—18 second category.

Representative sets of inter-response time distributions have been plotted in Fig. 5. In control sessions both animals showed a tendency

to emit responses with either short inter-response times (in the 0—3 second category) or ones that approximated the requirements of the schedule (i.e., only inter-response times of 15 seconds or greater were reinforced). The dose of 1.0 mg/kg amphetamine had little effect on the distributions; both rats continued to show the "timing" of responses indicated by the relatively high proportion of responses in the 12—18 second range,

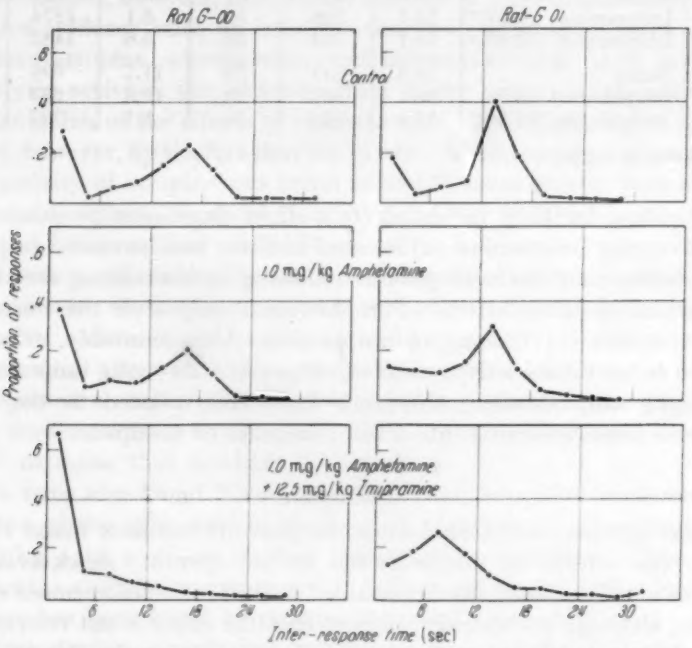

Fig. 5. Inter-response time distributions for two animals following control, amphetamine, and amphetamine + imipramine injections. Each distribution was taken from a single session

whereas amphetamine in combination with 12.5 mg/kg imipramine, itself ineffective, disrupted this pattern of response emission. In the case of G-01 the modal value shifted to the 6—9 second category, whereas all indications of "timing" behavior were obliterated in the case of G-00.

The averages of the median inter-response times, of the total numbers of responses in the reinforcement periods, and of the time-out responses have been tabulated in Table 3. The data for G-00 indicate that (1) both doses of imipramine + saline produced a slight depression of behavior (as indicated by higher medians and fewer responses), as was the case in the avoidance experiments, (2) amphetamine produced a moderate increase in behavioral output (lower medians and an increased number of both types of responses), which was (3) augmented by the concurrent administration of imipramine.

Table 3. *Effects of imipramine and/or amphetamine on total responses (RS), time-out responses (T.O.), and inter-response time medians (MDN)*

Rat	First injection	Second injection					
		Saline			Amphetamine (1.0)*		
		MDN	*RS*	*T.O.*	*MDN*	*RS*	*T.O.*
G-00	Saline	13.6	702	61	8.1	857	176
	Imipramine (6.25)*	15.7	526	38	5.1	1874	601
	Imipramine (12.50)	15.1	594	30	5.6	1482	822
G-01	Saline	14.8	511	59	11.7	698	8
	Imipramine (6.25)	16.1	443	29	12.8	233	10
	Imipramine (12.50)	15.8	513	59	8.1	727	137

* Dose in mg/kg.

The data for G-01 indicated (1) a slight depression predominantly at 6.25 mg/kg imipramine, (2) lowered medians and increased responses only during reinforcement periods following amphetamine, and (3) an augmentation of the effects of amphetamine only after the concurrent administration of 12.5 mg/kg imipramine. Unaccountably, there appeared to have been a depression of output at 6.25 mg/kg imipramine + 1.0 mg/kg amphetamine; this may have been related to the slight decrease noted following this dose of imipramine + saline.

Discussion

Like atropine and scopolamine, imipramine has been found to augment the effects of amphetamine in an operant, shock-avoidance situation. The related effects obtained in the food-reinforcement experiments, although preliminary, suggest that the effect is not restricted to an avoidance situation. Further, a comparison of the dose-response curve for imipramine + saline with that for imipramine + amphetamine indicates a true potentiation of the effects of amphetamine by imipramine.

SIGG has suggested that imipramine may exert its effects by "sensitization of adrenergic synapses." In his discussion of SIGG's paper, ROTHLIN pointed out that the antagonism of parasympathetic and sympathetic systems may be operative centrally as well as peripherally and that "anticholinergic and adreno-sensitizing actions are synergistic in their end-effect." Thus, "sensitization" of sympathetic systems could arise as a result of the parasympatholytic activity of a compound. Further, the effects of sympathomimetics would presumably be augmented by such parasympatholytic action.

Our own preliminary interpretation of the interaction of the anticholinergics with amphetamine incorporates a point of view not unlike that of ROTHLIN. We have previously discussed (CARLTON and DIDAMO) data that strongly suggest that the interaction of atropine and amphet-

amine occurs centrally rather than peripherally. Further, this interaction might be interpreted as reflecting a normal antagonism of adrenergic and cholinergic systems analogous to that which is known to occur peripherally. Since sympathomimetics in general appear to increase response output, we suppose that some cholinergic system normally plays an inhibitory role that is attenuated by scopolamine and atropine. It should be emphasized, however, that, in the absence of a great deal more corroborative evidence, such an interpretation is a purely speculative one.

Since atropine, scopolamine, and imipramine each have parasympatholytic activity, this shared action might explain their individual augmentation of the effects of amphetamine. This possibility is contradicted, however, by the fact that the *in vitro* (rabbit ileum) parasympatholytic activity of atropine was found to be 157 times greater than that of imipramine (SIGG). Further, HOROVITZ and CHOW have recently found that the "dissociation" of EEG effects and behavior observed with atropine (e.g., WIKLER; BRADLEY and ELKES) does not appear to be characteristic of imipramine. In cats with chronically implanted electrodes, a dose of imipramine (5.0—10.0 mg/kg, i.p.) that produced EEG-slowing also produced a clear-cut behavioral depression. (SIGG has reported a similar behavioral depression.) Atropine, on the other hand, does not appear to alter behavior, and may even produce "excitement" at doses that produce EEG-slowing.

We have also found (CARLTON, 1961) that, in a food-reinforcement situation of the type used in the present experiments, atropine (2.0—6.0 mg/kg, i.p.) produces marked increases in responding. Thus both imipramine and atropine augment the effects of amphetamine but *only* atropine has been found to produce increased responding.

These studies suggest that imipramine does not strictly mimic the central effects of atropine. It seems unlikely, therefore, that the qualitative similarity of the effects of atropine or imipramine in combination with amphetamine can be attributed to identical mechanisms. We feel that it is more tenable to suppose that the potentiation of the action of amphetamine by atropine may be related to its parasympatholytic action, whereas the potentiation by imipramine is due to some other, as yet undetermined, mechanism. The behavioral data reported here can, nonetheless, be taken as corroborating SIGG's notion that the effects of imipramine are mediated by an unspecified "sensitization" of adrenergic systems.

Summary

The effects of imipramine in combination with amphetamine were studied in two operant-behavior situations. In one of these, rats were required to press a response lever in order to avoid brief electric shocks; in the other, rats were reinforced for displacing a small response "key"

only if such responses were emitted at a rate no greater than one per 15 seconds.

In general, imipramine was found to potentiate the tendency of amphetamine to increase responding in these situations. The relationship of imipramine to atropine, which has a similar action in combination with amphetamine, is discussed.

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Augmentation of the Behavioral Effects of Amphetamine by Scopolamine

By

PETER L. CARLTON

With 2 Figures in the Text

(Received March 6, 1961)

We have previously reported (CARLTON and DIDAMO) that the action of *d*-amphetamine in increasing the rate of responding in an operant, shock-avoidance situation is augmented by low, non-effective doses of atropine. The present supplementary study was designed to determine whether the related parasympatholytic, scopolamine, would similarly interact with amphetamine.

Methods

The apparatus was a sound-insulated, ventilated response chamber which contained a response lever and a grid floor through which 1.0 to 1.5 ma electric shocks of 0.25 sec could be delivered. Depression of the lever activated an electric circuit that recycled an electric timer set for 20 sec. A brief shock was delivered to the animal after each 20 sec interval during which no response had occurred. This shock-avoidance procedure has been described in detail elsewhere (SIDMAN). Electrical impulse counters and a Gerbrands Cumulative Recorder registered all responses and shock-deliveries.

Each of the adult, male, albino rats used as subjects was given about 35 hours of exposure to the shock-avoidance schedule. After the level of performance had stabilized, experimental sessions ensued in which the behavioral effects of scopolamine alone were evaluated. From the resultant dose-response curves the non-effective doses of scopolamine used in the present study were estimated for each rat.

Each rat was then given 2—3 sessions in which the effects of various doses of *d*-amphetamine were evaluated. From these evaluations a dose of amphetamine that produced moderate and reliable increases in responding was selected. The dose selected for each rat was used throughout the present experiment.

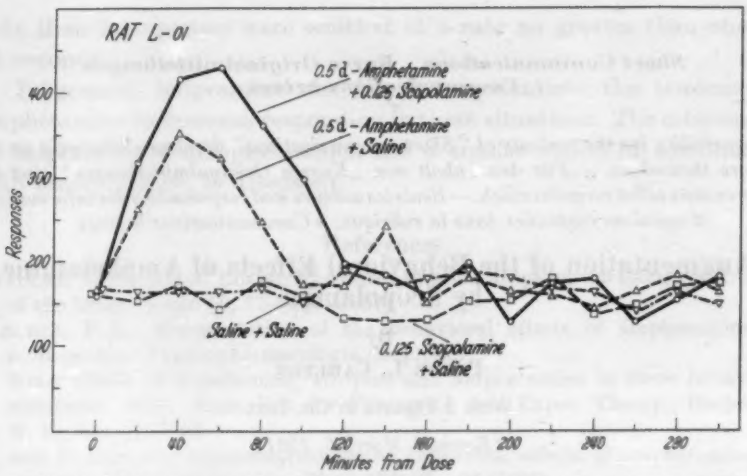

Fig. 1. Numbers of responses in each of the successive 20-minute intervals of the post-dose period. Each curve was plotted from data recorded in single sessions for one animal

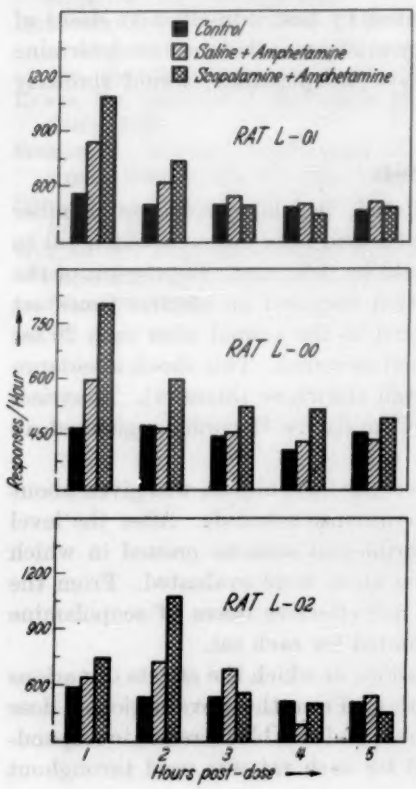

Fig. 2. The mean numbers of responses in each of the 5 successive hours of the post-dose period for 3 animals. ■ Control; ▨ Saline + Amphetamine; ▩ Scopolamine + Amphetamine

In the studies of scopolamine in combination with amphetamine, each animal was given 1 hour of exposure to the shock-avoidance schedule, removed and given 2 intraperitoneal injections (at a constant volume of 1 cc/kg each), and replaced in the apparatus for an additional 5 hours. In each of these sessions, one of the following pairs of doses was given: saline + saline (control), saline + *d*-amphetamine sulfate, scopolamine hydrobromide + saline, or scopolamine + *d*-amphetamine. Each rat underwent 1 or 2 sessions per week. Each of the pairs of doses was given in random order; drug doses were at least one week apart. The effects of each of the dose combinations

were evaluated in 2—4 determinations for each rat. The doses of the scopolamine and *d*-amphetamine salts for the individual rats L-00, L-01 and L-02, on a mg/kg basis, were 0.06 and 0.5, 0.125 and 0.5, and 0.125 and 2.0, respectively.

Results

The numbers of responses emitted in single sessions at each drug combination for one animal have been plotted in Fig. 1. The values plotted at 0 were taken from the last 20 minutes of the pre-dose period. Neither saline + saline nor scopolamine + saline altered the rate of avoidance responding that characterized the last 20 minutes of the pre-dose period. Saline + amphetamine, on the other hand, produced an increase in avoidance responding that was augmented by the concurrent administration of scopolamine. Note that the increases in responding had subsided in about 2 hours.

Fig. 2 summarizes the averaged response data for the three animals. In the first 2 hours, higher response rates were obtained after the saline + amphetamine and scopolamine + amphetamine injections than were obtained in the corresponding control periods. The increases in responding that followed scopolamine + amphetamine were uniformly greater than the increases obtained after the saline + amphetamine injections.

Statistical analysis[1] of the differences between the response levels observed after scopolamine + amphetamine and those levels observed after saline + amphetamine indicated that these differences were significant ($p < 0.01$) only for the first 2 post-dose hours. A similar analysis, which compared the response levels obtained after scopolamine + saline injections with those obtained in control sessions, indicated that scopolamine alone at these doses was ineffective in altering the rate of avoidance responding.

[1] We evaluated the difference between the effects of scopolamine + amphetamine and those of saline + amphetamine by assigning each animal a difference score obtained by subtracting the mean number of responses recorded in the first 2 hours after the saline + amphetamine injections from the corresponding number recorded after scopolamine + amphetamine. The mean of these scores was evaluated by a t-test under the hypothesis that the mean difference in the population was zero. The resultant t was 11.4. Analogous t values for post-dose hours 3 and 4 and for hour 5 were not significant. Two-hour periods were used because the time-course of drug effects differed from animal to animal; the 2-hour values incorporated the major differential drug effects for all animals. Similar t-values were used in comparing the scopolamine + saline and saline + saline data. These were all non-significant.

Comment

The finding that scopolamine augmented the effects of amphetamine was not unexpected because of our previous observation that the related parasympatholytic, atropine, also had this augmenting action. It seemed advisable, however, to perform the present supplementary study because a number of the central actions of scopolamine do not appear to be shared by atropine. The fact that both drugs did, in fact, augment the effects of amphetamine extends the generality of our previous finding with atropine and lends some support to the notion that this shared action is related to the parasympatholytic activity of the two drugs.

Summary

Non-effective doses of scopolamine were found to augment the tendency of amphetamine to increase the response rates of rats working in an operant, shock-avoidance situation. This finding is presumably related to our earlier observation that the related parasympatholytic, atropine, also had this augmenting action[2].

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1. La revue «Psychopharmacologia» a pour but la publication à bref délai de communications scientifiques relatives à l'analyse et à la synthèse des effets des drogues sur le «comportement» dans toute l'étendue du terme. Ces publications peuvent être d'ordre clinique; il peut s'agir également de recherches spécialisées dans les domaines de la psychologie expérimentale, de la neurophysiologie, de la neurochimie, de la pharmacologie et de disciplines apparentées.

2. La revue publie tous travaux originaux, communications cliniques, communications brèves, lettres à l'éditeur et bibliographies. Les revues générales ne pourront toutefois être publiées que sur demande de la Rédaction.

3. Le *contenu* des articles ne doit pas être publié ni être envoyé dans ce but à d'autres revues.

4. Les *manuscrits* doivent être rédigés en français, en anglais ou en allemand. Tout article doit contenir un bref résumé présentant les résultats essentiels; ce résumé sera rédigé en anglais pour les articles écrits en allemand ou en français et dans l'une des trois langues citées pour les articles rédigés en anglais.

5. Les articles traitant de travaux personnels contiendront une introduction courte mais précise au problème traité suivie de paragraphes relatifs à la méthode, résultats, discussion, résumé et bibliographie.

6. Le *texte* doit être aussi clair et aussi bref que possible. Le manuscrit doit être lisible, tapé à la machine (double interligne) sur le seul recto de la feuille. Les illustrations seront accompagnées de légendes concises et figureront sur des feuilles séparées.

7. Les *illustrations* seront limitées au strict nécessaire. Diagrammes et graphiques peuvent être présentés sous forme d'esquisses au crayon, la reproduction définitive étant assurée par l'éditeur. Les auteurs sont priés d'envoyer les photos originales sur papier glacé pour les reproductions en demi-ton. Un même résultat ne sera pas représenté simultanément sous forme de graphique et de tableau.

8. *Les références du texte* seront suivies du nom de ou des auteurs (éventuellement entre parenthèses) s'il n'y en a pas plus de deux. Dans les autres cas, le premier seul est cité suivi de «et al.» Si le même, ou les mêmes auteurs, sont plusieurs fois cités, on fera suivre le nom de la date de parution de chacune des œuvres nommées. Au cas où plusieurs ouvrages du ou des mêmes auteurs dateraient de la même année, on ajoutera à la date les lettres a, b, c, etc.

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Exemple: Dews, P. B.: Studies on behavior, IV. Stimulant actions of methamphetamine. J. Pharmacol. exp. Ther. **122**, 137—147 (1958a).

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Exemple: Bleuler, E.: Lehrbuch der Psychiatrie, 9. Aufl., Berlin-Göttingen-Heidelberg, Springer 1955.

9. Il sera fait de chacun des articles publiés 75 *tirés à part* mis à la disposition des auteurs à titre gratuit.

10. Les manuscrits originaux accompagnés d'un double doivent être envoyés

a) pour les U. S. A. et le Canada au
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b) pour les autres pays au
Prof. Dr. E. Rothlin, Sonnenweg 6, Bâle, (Suisse)

c) ou à tout autre membre de la rédaction selon le choix de l'auteur.

11. Les éditeurs et les rédacteurs prient instamment d'observer strictement ces directives aussi bien en ce qui concerne le caractère original du manuscrit que les détails techniques. La collaboration entre auteurs et éditeurs en sera ainsi facilitée et les délais de publication raccourcis.

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